

## **$\alpha$ 1-antitrypsin deficiency**

Catherine M. Greene<sup>1\*</sup>, Stefan J. Marciniak<sup>2</sup>, Jeffrey Teckman<sup>3</sup>, Ilaria Ferrarotti<sup>4</sup>, Mark L. Brantly<sup>5</sup>, David A. Lomas<sup>6</sup>, James K. Stoller<sup>7</sup> and Noel G. McElvaney<sup>1</sup>.

<sup>1</sup>Department of Medicine, Royal College of Surgeons in Ireland Education and Research Centre, Beaumont Hospital, Dublin 9, Ireland.

<sup>2</sup>Cambridge Institute for Medical Research, University of Cambridge, Cambridge, UK.

<sup>3</sup>Department of Pediatrics, Saint Louis University, Missouri, USA.

<sup>4</sup>Department of Internal Medicine and Therapeutics Pneumology Unit, University of Pavia, Italy.

<sup>5</sup> Division of Pulmonary, Critical Care and Sleep Medicine, University of Florida College of Medicine, Gainesville, Florida, USA.

<sup>6</sup>UCL Respiratory , Division of Medicine, University College London, London, UK.

<sup>7</sup>Education Institute and Respiratory Institute, Cleveland Clinic, Ohio, USA.

Correspondence to:

C.M.G.

cmgreene@rcsi.ie

## ABSTRACT

$\alpha_1$ -antitrypsin deficiency (A1ATD) is an inherited disorder caused by mutations in *SERPINA1*, leading to liver and lung disease. It is not a rare disorder but frequently goes underdiagnosed or misdiagnosed as asthma, chronic obstructive pulmonary disease (COPD) or cryptogenic liver disease. The most frequent disease-associated mutations include the S and Z alleles of *SERPINA1*, which lead to the accumulation of misfolded  $\alpha_1$ -antitrypsin in hepatocytes, ER stress, low circulating levels of  $\alpha_1$ -antitrypsin and liver disease. Currently, no cure for severe liver disease exists and the only management option is liver transplantation, when liver failure is life threatening. A1ATD-associated lung disease predominately occurs in adults and is caused principally by inadequate protease inhibition. Treatment of A1ATD-associated lung disease includes standard therapies that are also used for the treatment of COPD, in addition to the use of augmentation therapy (infusions of human plasma-derived, purified  $\alpha_1$ -antitrypsin). New therapies targeting the misfolded  $\alpha_1$ -antitrypsin or attempting to correct the underlying genetic mutation are currently under development.

$\alpha_1$ -antitrypsin is a serine proteinase inhibitor, produced principally by hepatocytes but also by neutrophils, monocytes and epithelial cells of the lung and gut. It is a major circulating antiprotease in humans and its key function is the regulation of the proteolytic effects of neutrophil elastase in the lung, which can lead to a range of consequences including inflammation and changes in the extracellular matrix (ECM). In addition,  $\alpha_1$ -antitrypsin is also an acute phase protein with anti-inflammatory and immuno-modulatory properties.

$\alpha_1$ -antitrypsin deficiency (A1ATD, Online Mendelian Inheritance in Man (OMIM) entry 107400) is a disorder caused by mutations in the *SERPINA1* gene and is inherited in an autosomal and codominant pattern, indicating that the two copies of the gene (alleles) are active and contribute to the genetic trait.<sup>1</sup> This gene locus was previously called the protease inhibitor (PI) locus and this nomenclature is still used to describe the different genotypes linked to mutation in *SERPINA1*. Over 150 mutations in *SERPINA1* have been described. The most common, non-disease causing and so-called 'normal' allele is referred to as the 'M' allele; the most frequent (>95% of patients with A1ATD) disease-associated *SERPINA1* mutations, are referred to as 'S' and 'Z' alleles. The Z allele leads to severe disease symptoms and is the most widely studied variant. S and Z alleles lead to aberrantly folded  $\alpha_1$ -antitrypsin and reduced circulating levels of  $\alpha_1$ -antitrypsin. Patients with A1ATD can be classified based on their genotype, which can be heterozygous (patients who have 2 different *SERPINA1* alleles, for example M and Z alleles (PI\*MZ and PI\*MS genotypes)) or homozygous (patients who have 2 identical *SERPINA1* alleles, for example 2 Z alleles (PI\*ZZ genotype)) for the disease alleles. Each copy of the allele will contribute to the disease phenotype.

A1ATD predisposes to liver and lung disease (Figure 1), but patients with A1ATD might also be affected with asthma, granulomatosis with polyangiitis and panniculitis. A1ATD-associated liver disease is the consequence of accumulation of misfolded  $\alpha_1$ -antitrypsin in the ER of hepatocytes leading to ER stress. Currently, no cure for severe liver disease exists aside from liver transplantation. In contrast, A1ATD-associated lung disease is due to the consequence of low or undetectable circulating  $\alpha_1$ -antitrypsin levels leading to inadequate proteinase inhibition resulting, for example, in

damage to the protective layer in the lung. Additionally, polymers of misfolded  $\alpha_1$ -antitrypsin, in particular the Z-type  $\alpha_1$ -antitrypsin in the circulation and in lung tissue, and endoplasmic reticulum (ER) stress in monocytes and neutrophils have a role in the inflammation observed in A1ATD-associated lung disease. A1ATD-associated lung disease shares many characteristics of emphysema, but the pathology differs. A1ATD is also a genetic cause of chronic obstructive pulmonary disease (COPD) and is responsible for 1-2% of COPD cases. Moreover, the PI\*MZ genotype is a risk factor for the development of COPD. Individuals with A1ATD-associated lung disease receive many standard therapies that are used for the treatment of COPD, in addition to augmentation therapy using human plasma-derived, purified  $\alpha_1$ -antitrypsin to supplement the low levels of active  $\alpha_1$ -antitrypsin. New therapies that target the misfolding of mutant forms of  $\alpha_1$ -antitrypsin, or attempt to correct the underlying genetic mutation are being developed.

In this Primer, we summarize the epidemiology of A1ATD, discuss the pathophysiology of A1ATD-associated lung and liver complications and review current research in this field. We also consider existing treatment options for A1ATD and future developments that might further improve the outlook for patients with A1ATD.

## **[H1] Epidemiology**

A1ATD is a relatively common, disorder<sup>2,3</sup>. Although most prevalent in European (including Scandinavia, Spain and Portugal) and North American countries, A1ATD occurs world-wide. A1ATD has been reported in virtually all racial subgroups studied in almost 70 countries in 11 geographic regions around the world<sup>4</sup>. The global number of individuals with the PI\*MS or PI\*MZ genotype is 116 million and PI\*ZZ, PI\*SZ, or PI\*SS is 3.4 million. The prevalence of A1ATD has been estimated using two detection strategies: population-based screening and case reports (also called targeted detection). Many population-based screening studies for A1ATD have been performed<sup>4, 146</sup>, the largest two were performed in newborn infants in Sweden and Oregon and estimated a prevalence of PI\*ZZ of 1 per 1,639 individuals in Sweden ( $n=200,000$ ) and 1 per 5,097 individuals in Oregon ( $n=107,038$ )<sup>5</sup>. Data from one study suggests that approximately 100,000 individuals in the United States have A1ATD characterized by severely decreased serum levels<sup>6</sup>. Some targeted detection studies have assessed the prevalence of

A1ATD in individuals with several diseases. For example, one study estimated the prevalence of severe A1ATD in individuals with COPD as between 0–12%, with a mean value of 3.6%. In general, all data regarding the prevalence of A1ATD is likely confounded by the method of ascertainment and by general under-recognition of A1ATD (see Diagnosis, Screening and Prevention).

## **[H2] Genetic and environmental risk factors**

A genome wide association study tightly linked circulating  $\alpha_1$ -antitrypsin levels in a general population sample to the *SERPINA1* gene cluster<sup>7</sup>. Also, the detrimental role of the exposure to cigarette smoke on the clinical phenotype of A1ATD has been demonstrated<sup>8</sup>. In a Swedish study, all 35-year-old individuals with PI\*ZZ had normal liver and lung function but smoking frequency was significantly lower among patients with A1ATD, compared with controls<sup>9</sup>. Never-smokers with A1ATD also had abnormal chest X rays and lung function<sup>10</sup>. Nevertheless, the wide spectrum of clinical phenotypes associated with A1ATD could be caused by interactions between genetic and environmental factors other than *SERPINA1* and smoking alone. Indeed, single studies have identified potential genetic modifiers of COPD phenotypes in patients with severe A1ATD. For example, genetic variations in *MMP1/MMP3* and *TNF* have been associated with air transfer and chronic bronchitis, respectively, in patients with A1ATD<sup>11, 12</sup> and polymorphisms in *IL10*, *CHRNA3* and *IREB2* are associated with lung function in individuals with the PI\*ZZ genotype<sup>13, 14</sup>. The expression of the lung disease for example, ranges from asymptomatic to severe emphysema in individuals with PI\*ZZ. This heterogeneity is likely the result of genetic predisposition to lung disease and some environmental factors. For example, interplay between cigarette smoke-induced oxidative stress and Z-type  $\alpha_1$ -antitrypsin polymerization can impact on cellular inflammation and cytokine expression<sup>15</sup>. Regarding the role of the environment on A1ATD few data are available suggesting that outdoor air pollution can worsen respiratory status and predict lung function decline in individuals with PI\*ZZ<sup>16, 17</sup>. Another study revealed a significant interaction ( $P<0.0001$ ) between the PI\*MZ genotype and high levels of exposure to vapours, gases, dusts and fumes on annual change in FEF25–75% levels<sup>18</sup>. A separate study noted a significant interaction ( $P=0.03$ ) between high-level vapours, gases,

dusts and fumes and annual changes in FEV<sub>1</sub>/FVC<sup>17</sup>. Overall, larger annual declines in lung function in PI\*MZ carriers, compared to individuals with PI\*MM was associated with outdoor particulate matter of  $\leq 10 \mu\text{m}$  and FEF25-75% decline associated with vapours, gases, dust and fumes was observed only in ever smokers with the PI\*MZ genotype<sup>18</sup>. Unlike smoking<sup>19</sup>, environmental or passive tobacco smoke exposure is not a risk factor for PI\*MZ individuals<sup>18</sup>.

## **[H1] Mechanisms/pathophysiology**

The misfolding and accumulation of mutant forms of  $\alpha_1$ -antitrypsin within the ER of  $\alpha_1$ -antitrypsin-producing cells can lead to toxicity; this primarily affects the liver and to a lesser extent the lungs. The accumulation of misfolded  $\alpha_1$ -antitrypsin causes cellular damage through two principal mechanisms; the perturbation of homeostasis within the lumen of the ER and the assembly of polymers (especially of Z-type  $\alpha_1$ -antitrypsin) in the circulation, lumen of the lung or within tissues that can cause chemotaxis and/or activation of inflammatory cells<sup>20</sup>. Retention of mutant  $\alpha_1$ -antitrypsin in  $\alpha_1$ -antitrypsin-producing cells leads to low or undetectable  $\alpha_1$ -antitrypsin levels, which can cause defects in other tissues owing to a lack of proteinase inhibition, especially in the lung.

## **[H2] Genetic basis of disease**

$\alpha_1$ -antitrypsin is encoded by the *SERPINA1* gene (Figure 2). Different  $\alpha_1$ -antitrypsin transcripts are generated in different tissues owing to different transcription initiation sites, alternative splicing of untranslated exons<sup>21-23</sup> and tissue-specific proinflammatory cytokines in particular IL-6 and leukaemia inhibitory factor and the acute phase mediator oncostatin-M<sup>24-28</sup>. A specific quantitative reverse-transcriptase PCR test has been developed to quantify the expression of the different *SERPINA1* transcripts, to improve understanding of the regulatory mechanisms controlling *SERPINA1* expression<sup>29</sup>.

The *SERPINA1* gene is highly polymorphic and mutations in this gene cause a hereditary co-dominant autosomal disorder. Pathological *SERPINA1*  $\alpha_1$ -antitrypsin variants are classified as either 'deficient' or 'null'. Deficient variants occur as a result of

a point mutation that causes retention of the  $\alpha_1$ -antitrypsin protein in hepatocytes and other  $\alpha_1$ -antitrypsin-producing cells, resulting in low levels of  $\alpha_1$ -antitrypsin in plasma. Null mutations generally occur due to the presence of a premature stop codon and patients with these mutations have no detectable  $\alpha_1$ -antitrypsin in serum. The most common severely deficient variant is Z-type  $\alpha_1$ -antitrypsin and is caused by a single nucleotide polymorphism (SNP) in *SERPINA1* that results in the substitution of glutamic acid for lysine at residue 342 (Glu342Lys, reference SNP cluster ID (rs)28929474), which is found at a frequency between 2–5% in Caucasian individuals of European descent. At least 40 other deficient variants have been identified over the last few decades; these variants are rare, the molecular mechanism by which they cause disease varies and they can be prognostic for either liver and lung diseases<sup>30,31</sup>. Similarly, up to 34 null alleles have been characterized to date<sup>32</sup>, most of which are extremely rare.

## [H2] Mutant $\alpha_1$ -antitrypsin

$\alpha_1$ -antitrypsin's primary target protease is neutrophil elastase, but it can also inhibit other proteases.  $\alpha_1$ -antitrypsin uses the characteristic serpin inhibitory mechanism to irreversibly inhibit the activity of neutrophil elastase<sup>33</sup>.

The Z-type of  $\alpha_1$ -antitrypsin is retained within the ER of hepatocytes as ordered polymers that become sequestered in inclusions, which are Periodic Acid Schiff-positive, diastase-resistant on histological examination<sup>34, 35</sup>. This same process underlies the severe  $\alpha_1$ -antitrypsin plasma deficiency and intrahepatic inclusions of other protein variants of  $\alpha_1$ -antitrypsin; Siiyama (Ser53Phe)<sup>36</sup>, Mmalton ( $\Delta$ Phe52)<sup>37</sup>, and King's (His334Asp)<sup>35</sup>. Polymerisation also underlies the deficiency of the mild S (Glu264Val), I (Arg39Cys), Queen's (Lys154Asn) and Baghdad (Ala336Pro)<sup>38</sup> alleles of  $\alpha_1$ -antitrypsin. However the rate of polymer formation, which is proportional to the destabilising effect of the mutation on the protein<sup>39</sup>, is much slower for the **I, S, Queen's and Baghdad alleles** and explains the absence of liver disease and the association with only mild plasma deficiency.

The initial description of the polymers of Z-type  $\alpha_1$ -antitrypsin described a linkage between the reactive centre loop and  $\beta$ -sheet A on adjacent Z-type  $\alpha_1$ -antitrypsin

proteins<sup>34</sup> (Figure 3a). Alternative linkages of  $\alpha_1$ -antitrypsin polymerisation have been described in the crystal structures of a dimer of antithrombin (linkage by a  $\beta$ -hairpin of the reactive centre loop and strand 5A (Figure 3b)<sup>40</sup>) and a trimer of  $\alpha_1$ -antitrypsin (linkage by strands 1C, 4B and 5B (Figure 3c))<sup>41</sup>. The biophysical characteristics of polymers of  $\alpha_1$ -antitrypsin formed by refolding following denaturing by guanidine supports the  $\beta$ -hairpin and  $\beta$ -sheet A linkage<sup>42</sup>. Data from small-angle X-ray scattering suggests that trimers, tetramers, and pentamers of Z-type  $\alpha_1$ -antitrypsin all form ring-like structures, consistent with a linkage by strands 1C, 4B and 5B (Figure 3d)<sup>43</sup>. In patients, ring-shaped polymers of  $\alpha_1$ -antitrypsin are rarely seen in inclusions from the livers of individuals with PI\*ZZ<sup>34</sup>.

## [H2] Antitrypsin deficiency

Mutation in *SERPINA1* results in enhanced neutrophil elastase activity, which causes a plethora of effects that can contribute to the pathophysiology of A1ATD-associated lung disease. Enhanced neutrophil elastase activity has a range of consequences that lead to inflammation and an enhanced rate of neutrophil reactive oxygen species production (Figure 4). Excessive cleavage of molecules involved in the immune response (complement factors, immunoglobulins, antimicrobial peptides<sup>44</sup> and cell surface receptors such as C-X-C chemokine receptor type 1 (CXCR1) and monocyte differentiation antigen CD14<sup>45, 46</sup>), result in a decreased activation of monocytes (owing to reduced responsiveness to lipopolysaccharide (LPS)) and a decreased efficiency of phagocytosis by neutrophils — one of the hallmarks of acute inflammation. Reduced expression of low affinity immunoglobulin  $\gamma$  Fc region receptor type III-B on the neutrophil surface and increased chemotaxis of neutrophils in response to IL-8 and soluble immune complexes<sup>47</sup>, together with degranulation of secondary and tertiary granules further exaggerates the production of reactive oxygen species<sup>48</sup>. Increased cleavage of coagulation factors and extracellular matrix or connective tissue molecules (elastin<sup>49</sup>, collagen<sup>50</sup>, fibronectin<sup>51</sup> and proteoglycans<sup>52</sup>), can also contribute to the pathology. Interestingly, neutrophil elastase can also induce gene expression of ECM modulators (matrix metalloproteases and cathepsins) by activation of disintegrin and metalloproteinase domain-containing protein 17 (ADAM17) and meprin A-mediated



epidermal growth factor receptor (EGFR) signalling<sup>53-56</sup>. Inactivation of tissue inhibitors of metalloproteases<sup>57</sup>, secretory leucoprotease inhibitor (SLPI)<sup>44</sup>, elafin<sup>58</sup> and cystatin-C<sup>59</sup> has also been reported. Other outcomes that occur either directly or indirectly due to decreased  $\alpha_1$ -antitrypsin levels in the lung, include goblet cell (mucus-secreting cells) hyperplasia, increased mucus secretion and impaired mucociliary clearance leading to defective bacterial clearance.<sup>60</sup> Moreover, reduced inhibition of caspase-3 can promote apoptosis of lung endothelial cells<sup>61</sup>. Emerging data have also indicated that leukotriene B4 production, and leukotriene B4 receptor 1 (BLT1) membrane receptor expression<sup>62</sup> are increased in neutrophils, as are tumour necrosis factor (TNF)- $\alpha$ -mediated peripheral blood neutrophil apoptosis<sup>63</sup>. Other evidence has demonstrated increased phosphorylation of p38 mitogen-activated protein kinases and NF $\kappa$ B inhibitor  $\alpha$  (I $\kappa$ B $\alpha$ ), and induction of matrix metalloproteinases and cytokines via serine/threonine-protein phosphatase 2A<sup>64</sup>.

## **[H2] Accumulation of $\alpha_1$ -antitrypsin in ER**

**[H3] Intracellular disposal mechanisms for misfolded  $\alpha_1$ -antitrypsin.** The retention of some protein variants, such as the Z-type  $\alpha_1$ -antitrypsin in hepatocytes is the inciting event in the pathophysiology of A1ATD-associated liver disease (Figure 5)<sup>65</sup> and leads to apoptosis and oxidative stress of hepatocytes. In healthy cells, misfolded proteins accumulated in the ER are degraded by the ubiquitin proteasome system — a process called ER-associated protein degradation (ERAD) — or by macroautophagy. In A1ATD, the increased load of misfolded protein in the ER causes excessive activation of these cellular disposal mechanisms. Soluble Z-type  $\alpha_1$ -antitrypsin is monitored in the ER and diverted to the ERAD pathway, whereas Z-type  $\alpha_1$ -antitrypsin polymers are degraded by macroautophagy. Much of the work investigating handling of misfolded  $\alpha_1$ -antitrypsin has concentrated on the Null Hong Kong (NHK) variant of  $\alpha_1$ -antitrypsin. The ERAD pathway is the major pathway for degradation of NHK  $\alpha_1$ -antitrypsin owing to its inability to fold,<sup>66</sup> but even polymerogenic mutants of  $\alpha_1$ -antitrypsin are also targeted for degradation by the ERAD pathway, despite having near-native conformations<sup>66, 67</sup>. The Z variant of  $\alpha_1$ -antitrypsin folds more slowly than M  $\alpha_1$ -antitrypsin and can adopt an

intermediate conformation; both of these factors might contribute to the targeting of Z-type  $\alpha_1$ -antitrypsin to the ERAD pathway<sup>35, 68, 69</sup>.

Glycoproteins (such as  $\alpha_1$ -antitrypsin) undergo cycles of N-glycan modification whilst within the ER. This mechanism acts as a timer to identify proteins failing to fold in an appropriate time. Abnormalities in the enzymes involved in this process might have a role in  $\alpha_1$ -antitrypsin misfolding in A1ATD. Experimental overexpression of ER mannosyl-oligosaccharide 1,2- $\alpha$ -mannosidase (ERManI), an enzyme that trims mannose residues from N-glycans, accelerates degradation of both NHK and Z-type  $\alpha_1$ -antitrypsin variants *in vitro*<sup>70, 71</sup>, whereas inhibition of ERManI stabilises the variants<sup>72</sup>. Interestingly, a less-frequent allele variant of *MAN1B1* (encoding ERManI) that is associated with reduced ERManI expression has been reported more frequently than expected in children requiring transplantation for Z-type  $\alpha_1$ -antitrypsin associated liver disease, suggesting that protein glycosylation has a role in  $\alpha_1$ -antitrypsin accumulation in patients<sup>73</sup>.

Whole organelles (for example, the ER) or large protein aggregates can be degraded by macroautophagy. This process involves engulfment of the structures by endomembranes that form autophagosomes, which fuse with the lysosome so that the contents are hydrolysed and degraded. Both *in vitro* and mouse models support a role for autophagy in the degradation of Z-type  $\alpha_1$ -antitrypsin<sup>66</sup>. Treatment of mice used to model A1ATD with carbamazepine (an autophagy-enhancing drug) reduces the accumulation of Z-type  $\alpha_1$ -antitrypsin protein in the liver<sup>74, 75</sup>. It is currently unknown whether macroautophagy shows selectivity for ER containing polymers of  $\alpha_1$ -antitrypsin, or if the turnover of ER in general is just increased.

**[H3] Endoplasmic reticulum stress.** When misfolded proteins accumulate within the ER and threaten to precipitate the cell is said to experience ER stress. ER stress triggers an unfolded proteins response (UPR) that reduces the influx of nascent proteins into the ER and simultaneously reprograms the cell to fold or dispose of the misfolded proteins more efficiently (Figure 6). This process involves the detection of ER stress by three ER stress sensors<sup>76, 77</sup>.

The misfolding variants NHK and Saar  $\alpha_1$ -antitrypsin, which are truncated and unable to fold, can trigger the UPR if expressed even at low levels<sup>78-83</sup>. All newly synthesized proteins sequester chaperones (including 78 kDa glucose-regulated protein (also known as BiP)) to keep them in a competent state for subsequent folding or direct them to the ERAD pathway if folding is impaired. NHK and Saar variants are normally efficiently degraded by the ERAD pathway, but if allowed to accumulate will sequester large numbers of BiP, leading to ER stress. The mechanism by which ER stress sensors are activated is debated. One model suggests the sequestration of BiP by accumulated misfolded proteins reduces the level of free BiP, leading to activation of ER stress sensors.<sup>84</sup> An alternative model suggests that the ER stress sensors interact directly with stretches of misfolded protein<sup>85</sup>.

Interestingly, the dramatic accumulation of polymeric  $\alpha_1$ -antitrypsin does not activate the UPR in most circumstances, despite ER stress<sup>82, 83, 86-90</sup>. As  $\alpha_1$ -antitrypsin polymers are generally thought to be relatively well-folded structures, they might not present misfolded stretches of amino acids and so fail to activate the ER stress sensors. However, the accumulation of polymers does seem to sensitize the cell to second insults that can cause ER stress<sup>83, 86-88</sup>. The mechanism for this sensitization is not well understood, but might involve altered protein mobility in the ER lumen, either owing to local alterations in viscosity or on the degree of ER interconnectivity<sup>83</sup>.

Accumulation of  $\alpha_1$ -antitrypsin polymers can impact on a variety of intracellular signalling pathways leading to the transcriptional upregulation of proinflammatory genes, including increased basal and LPS-induced expression of *IL6* and *CXCL8* in monocytes derived from patients with Z-type A1ATD, versus non- $\alpha_1$ -antitrypsin deficient individuals. Upregulation of proinflammatory genes is associated with intracellular accumulation of Z-type  $\alpha_1$ -antitrypsin polymers in monocytes<sup>91</sup>. The accumulation of polymerogenic  $\alpha_1$ -antitrypsin triggers nuclear factor  $\kappa$ B (NF- $\kappa$ B) signalling, which has been termed the 'ER overload response'. Little is known about this response, but leakage of calcium from the ER might have a role in NF- $\kappa$ B activation; chelation of cytosolic calcium has been shown to limit the activation of NF- $\kappa$ B<sup>88</sup>. In contrast, primary bronchial epithelial cells expressing low levels of Z-type  $\alpha_1$ -antitrypsin without polymer formation, also showed enhanced basal NF- $\kappa$ B signalling<sup>89</sup>. This indicates that increased NF- $\kappa$ B

signalling is not restricted to activation of the ER overload response. A possible alternative mechanism might involve the increased activity of ADAM17. Primary bronchial epithelial cells isolated from individuals with PI\*ZZ show hyperactive extracellular signal-regulated kinase (ERK) signalling, which depends on the activity of ADAM17. Moreover, increased ADAM17 activity has been reported on the surface of neutrophils of patients with A1ATD.

## **[H2] Extracellular $\alpha_1$ -antitrypsin polymers**

Polymers of  $\alpha_1$ -antitrypsin can also be detected in the blood<sup>92</sup>, bronchoalveolar lavage fluid and lung tissue of patients with A1ATD<sup>93,94</sup>. It is unclear if most polymers are formed by the polymerization of secreted Z-type  $\alpha_1$ -antitrypsin proteins in the extracellular space, because they can also be secreted at low levels by from dying cells<sup>95</sup>. However, most extracellular polymers are of hepatic origin, as the circulating levels of these polymers falls to undetectable levels four days after liver transplantation<sup>92</sup>.  $\alpha_1$ -antitrypsin can also be synthesised locally by airway epithelial cells, but at levels too low to allow the formation of intracellular polymers<sup>89</sup>.

Extracellular polymers of  $\alpha_1$ -antitrypsin have pro-inflammatory effects. For example, they are chemotactic and stimulatory for neutrophils and likely contribute to both pulmonary inflammation and the increased incidence of vasculitis or panniculitis seen in individuals with the PI\*ZZ genotype, following the deposition of these polymers in other tissues<sup>96</sup>.

## **[H1] Diagnosis, screening and prevention**

$\alpha_1$ -antitrypsin deficiency is a widely under-diagnosed condition. The estimate of the mean interval between the development of the first symptom of disease (usually dyspnoea (shortness of breath)) and initial diagnosis has not changed over the past 20 years and ranges from 5.6–8.3 years<sup>3,97</sup>. Similarly, the number of healthcare providers that patients with A1ATD see before the diagnosis is initially made has not lessened over time<sup>2</sup>. This delay in diagnosis has been associated with adverse psychosocial effects<sup>3</sup> and delays the implementation of disease management and the identification of family

members at risk of A1ATD. Thus, better recognition of this disorder by healthcare providers is urgently needed.

Under diagnosis of A1ATD is supported by three lines of evidence. Firstly, in all countries where diagnosis of A1ATD has been examined, only a small minority of the actual number of individuals with A1ATD have been diagnosed with this disorder<sup>98</sup>. Secondly, few physicians comply with the American Thoracic Society and European Respiratory Society (ATS/ERS) guidelines that state that all patients with COPD should be assessed for A1ATD<sup>99</sup>. Thirdly, patients with A1ATD frequently experience long delays and see many healthcare providers between their first symptom and diagnosis of A1ATD.

## [H2] Diagnostic tests

Owing to the heterogeneous clinical presentation, the diagnosis of A1ATD is predominantly based on the results of laboratory testing, although clinical features of  $\alpha_1$ -antitrypsin deficiency may be useful for selecting individuals for testing (Figure 7). The diagnostic test are well established throughout the world and involve the quantification of either plasma or serum  $\alpha_1$ -antitrypsin levels typically performed using a nephelometer, in combination with either genotyping or PI typing<sup>100</sup>. Genotyping usually starts off with the detection of the most common disease-associated alleles (Z and S) using genotype-based allele specific amplification by qPCR. This genotyping can be performed using DNA isolated from dried blood spots, whole blood and saliva and results from this diagnostic test dictate what subsequent diagnostic tests are performed (for example, Figure 7)<sup>101</sup>. Reflex testing, meaning, follow up testing for identification of alleles other than S or Z, is usually performed by protease inhibitor typing using isoelectric focusing of serum or plasma. Each phenotype of A1ATD displays a characteristic banding pattern on isoelectric focusing that can be compared to known reference samples<sup>100</sup>. Although S and Z alleles are present in >95% of all patients with A1ATD, the remaining 5% have rare deficiency alleles, such as those associated with reduced, dysfunctional or absent plasma  $\alpha_1$ -antitrypsin levels<sup>102</sup>. These rare alleles are not detected by routine diagnostic methods,

but require a combination of protease inhibitor typing and next generation sequencing of *SERPINA1*<sup>102</sup>.

## **[H2] Patient selection for A1ATD diagnostic testing**

Three approaches to select individuals for diagnosis testing of A1ATD exist. First, the diagnostic testing of individuals with symptoms or signs consistent with A1ATD, such as early onset, primarily lower lobe emphysema. In the past, this paradigm led to under diagnosis and late diagnosis of A1ATD. Second, predisposition testing of those who might be high-risk for A1ATD, such as asymptomatic individuals carrying a genetic mutation in *SERPINA1* and who have low levels  $\alpha_1$ -antitrypsin levels and a family member with A1ATD. Development of symptoms is likely for these patients, but it is not certain. Third, targeted detection in patients with a clinical reason to suspect A1ATD. Including, all patients with conditions associated with increased prevalence of A1ATD, such as COPD, poorly responsive asthma, cryptogenic liver disease, granulomatosis with polyangiitis, bronchiectasis of unknown aetiology and panniculitis, in addition to first degree relatives of patients with A1ATD. Targeted detection is similar to diagnostic testing but applies the ATS/ERS guidelines for the diagnosis and management of patients with A1ATD and has been shown to substantially increase the rates of diagnosis<sup>103</sup>. The use of the ATS/ERS criteria for targeted detection has been shown to enrich the detection of A1ATD; the allele frequency for the Z allele was over fourfold higher in the targeted population, compared with an unselected sample of the general population<sup>103</sup>. Guidelines regarding the potential benefits of targeted detection versus screening should be revisited particularly in lieu of the increased understanding of the pathogenesis of A1ATD-associated-disease.

## **[H2] Screening**

Screening guidelines for A1ATD are dynamic and rapidly evolving. The ATS/ERS guidelines do not recommend population-wide neonatal screening for A1ATD<sup>99</sup> (the testing of groups without known risk factors). This is based on evidence from a Swedish study<sup>104</sup>, which showed that although neonatal screening reduced parental smoking rates following detection of a genetic mutation in *SERPINA1*, there was an increased incidence

of parental distress and a negative impact on the mother–child relationship<sup>104</sup>. Also, the ATS/ERS guidelines do not generally recommend universal screening of adolescents aged <11 years, but suggest that screening should be discussed with individuals in areas with a high prevalence of A1ATD or if parental smoking rates are high, providing that adequate genetic counselling is given. Recommendations for adults are similar to those for adolescents<sup>99</sup>. The ATS/ERS guidelines<sup>99</sup> recommend testing high-risk groups, including all individuals appropriate for targeted detection.. This screening approach has been shown to increase the detection of  $\alpha$ 1ATD.

The 2014 Global Initiative for Chronic Obstructive Lung Disease (COPD) recommendations<sup>105</sup> quote the World Health Organization<sup>106</sup>, who recommend that COPD patients from areas with a particularly high prevalence of  $\alpha_1$ -antitrypsin deficiency should be tested for A1ATD. Also, they noted that individuals with COPD with  $\alpha_1$ -antitrypsin deficiency present with panlobular, lower lobe emphysema (compared to centrilobular apical distribution in COPD) at a younger age (<45 years), compared to patients with COPD and suggest that family members at risk of  $\alpha_1$ -antitrypsin deficiency should be identified. The Global Initiative for COPD recommendations are not dissimilar from those that led to substantial under diagnosis of  $\alpha$ 1ATD for the past 50 years.

## **[H2] A1ATD registries**

Data from rare disease patient registries increases awareness and knowledge of rare diseases, and is important for supporting both clinical and epidemiological research and monitoring of orphan drugs and the use of off-label medications<sup>107</sup>. Moreover, these registries are important for patients and their families; they provide a beneficial effect on health and social services planning and the ability to improve quality of care, quality of life and patient survival. According to the European Organization for Rare Diseases, the ideal patient registry should be disease-centred, be able to be used in combination with other data sets, contain minimum set of common data elements, be sustainable through the encouragement of public-private partnerships, should include data from various

sources (patients and healthcare professionals) and link with corresponding biobank data., Currently, no A1ATD registries exist that meets these criteria.

The first prospective registry for patients with A1ATD was the National Heart, Lung and Blood Institute (NHLBI) Registry which enrolled 1,129 patients with severe A1ATD between 1989-1992 and followed them until 1996<sup>108</sup>. This Registry collected information on patient demographics, medical history, measurements of pulmonary function and other laboratory evaluations at baseline and at 6-month or yearly intervals during follow-up. Data generated from this registry has produced some of the pivotal findings on the natural history of A1ATD, patient mortality and problems associated with a delayed diagnosis. Also, the effects of  $\alpha_1$ -antitrypsin augmentation therapy for patients was examined, although it was recognized that these results needed to be viewed with circumspection as results were not generated from a randomized clinical trial.

The current Alpha-1 Foundation Research Registry for patients in the US commenced enrolment in 1997 and enrolled patients with mildly-deficient genotypes between 1997-2002<sup>109</sup>. This registry is a contact registry, allowing appropriate patients with A1ATD to receive invitations to participate in clinical trials, although plans are to enlarge the remit of this registry. An Alpha One International Registry was also founded in 1997, which aims to establish an international database of patients with A1ATD to collect data on demographics, to promote and coordinate basic and clinical research into  $\alpha_1$ -antitrypsin deficiency, to collect, assess and disseminate information regarding different aspects of  $\alpha_1$ -antitrypsin deficiency and also, to raise support and awareness of  $\alpha_1$ -antitrypsin deficiency. The Alpha One International Registry now includes almost twenty European and non-European countries<sup>110</sup>. There is only one inclusion criterion for the international registry, in that the patients must have a PI\*ZZ, PI\*SZ or other severely deficient phenotype. Some patients (for example patients in certain national registries) received annual follow up to collect information allowing the documentation of disease characteristics, treatments, in addition to the smoking habits and lung and liver function of patients. There are also other large non-affiliated registries for  $\alpha_1$ -antitrypsin deficiency<sup>103, 111</sup>.

## **[H2] Prevention**



There are compelling reasons to identify patients with A1ATD early, for example to allow patients access to specific therapies and improve opportunities to avoid the environmental triggers of lung disease, including by the avoidance of personal and passive cigarette smoking<sup>112-114</sup>.

Personal cigarette smoking is associated with a considerable reduction in the life span of patients with A1ATD<sup>115</sup> and one study has shown that never-smokers with A1ATD might have normal life spans<sup>115</sup>. Importantly, patients with A1ATD who have developed COPD do so following exposure to a much lower number of pack-years of cigarette smoking, compared with individuals with COPD in the absence of A1ATD.

The early identification of  $\alpha_1$ -antitrypsin deficiency in neonates, adolescents and adults is associated with a reduction in the number of patients electing to start smoking with lower addiction rates as a consequence, and also, an increase in smoking cessation rates<sup>116, 117</sup>. For these reasons, counselling on the avoidance or cessation of smoking should be the number one focus for physicians and health care providers following the identification of  $\alpha_1$ -antitrypsin deficient patients of any age.

In addition to exposure to cigarette smoke, some occupational exposures such as mineral dust and certain fumes are associated with increased impairments in lung function and symptoms of respiratory disease in patients with A1ATD<sup>118</sup>.

Modifiable risk factors for liver disease are less well understood, but obesity is known to increase the risk. In addition, males are more at risk of liver disease, compared to females<sup>119</sup>. Current recommendations for patients with A1ATD include vaccinations for hepatitis A and B and moderate alcohol consumption and a healthy diet in individuals with PI\*ZZ<sup>112</sup>.

## [H2] Monitoring A1ATD-associated complications

[H3] Monitoring lung complications. Spirometry (lung function tests) typically seen in patients with A1ATD who have emphysema include a reduced forced expiratory volume in 1 second (FEV<sub>1</sub>), reduced FEV<sub>1</sub>/forced vital capacity ratio, air trapping (raised residual volume/total lung capacity ratio) and a low diffusion capacity. A partial reversibility of the airflow obstruction (as defined by an increase of 12% or 200 ml in FEV<sub>1</sub> after a

bronchodilator) is common in individuals with COPD secondary to A1ATD. Beyond this CT is a good parameter for lung diagnosis and has been used in various A1ATD augmentation therapy trials.

[H3] Monitoring liver complications. Many authorities, such as the American Association for the Study of Liver Disease, suggest regular monitoring (at least once per year) of patients with A1ATD for the development of liver disease, by a physician familiar with liver disease and associated complications<sup>120</sup>. Monitoring should include history and physical examination sensitive for liver disease, such as focusing on the detection of splenomegaly, and laboratory assessment of white blood cell count, platelet count, levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, albumin, bilirubin and assessment of the international normalized ratio. Progressive liver disease is often accompanied by granulocytopenia, thrombocytopenia and elevated liver enzymes and bilirubin and coagulopathy. As with the detection of other liver diseases, a baseline liver ultrasonography is often considered useful and is recommended every 6 months for patients at increased risk (>2% per year) hepatocellular carcinoma, in guidelines issued by the American Association for the Study of Liver Disease (AASLD)<sup>121</sup>. Although data for the risk of hepatocellular carcinoma in  $\alpha_1$ -antitrypsin deficiency is lacking, the AASLD guidelines should be applied to patients with A1ATD with evidence of cirrhosis, portal hypertension or persistently large elevations of liver tests.

## **[H1] Management**

### **[H2] Lung disease**

The 2011 Global Initiative for COPD (GOLD) strategy can identify patients with A1ATD at increased risk of poorer outcomes from lung disease, specifically focusing on mortality, lung function decline and exacerbations<sup>122</sup>. In the Swedish registry of individuals with PI\*ZZ, respiratory symptoms were the most common presenting feature A1ATD (in 43% of patients) and respiratory disease was the most common cause of death (55% of patients)<sup>10</sup>. The presence of lung disease is dependent on the smoking

history of the patient; in never-smokers 28% of patients fulfilled spirometric criterion for COPD, which rose to 72% in ex-smokers.

The rationale for the treatment of  $\alpha_1$ -antitrypsin deficiency-associated lung disease is to increase the levels of  $\alpha_1$ -antitrypsin in the lung towards levels seen in healthy individuals (that is, augmentation therapy), thus reversing the lack of inhibition of neutrophil elastase and other proteases, which, when uninhibited, can cause emphysema. Initially, augmentation therapy was performed by the intravenous delivery of plasma-purified  $\alpha_1$ -antitrypsin to patients<sup>123</sup>, which resulted in increased levels of  $\alpha_1$ -antitrypsin and increased inhibition of neutrophil elastase both in serum and on the pulmonary epithelial surface. Data from a number of clinical trials have shown a clinical benefit of the use of augmentation therapy for the treatment of A1ATD (Table 1). Augmentation therapy is usually commenced with evidence of lung disease (usually equating to a decreased FEV1 and is administered once per week as intravenous infusions.

Concerns about the purity of human plasma-derived purified  $\alpha_1$ -antitrypsin and the transmissibility of infection has led to the evaluation of transgenic and recombinant sources of  $\alpha_1$ -antitrypsin. Recombinant  $\alpha_1$ -antitrypsin has been successfully produced in bacteria, yeast and transgenic sheep (engineered to produce  $\alpha_1$ -antitrypsin in their milk), but; these recombinant proteins have lack of, or abnormal glycosylation, which causes altered renal clearance and a short half-life following intravenous administration<sup>124</sup>. Clinical trials investigating the use of an inhaled, recombinant form of  $\alpha_1$ -antitrypsin that has an appropriate pulmonary half-life are currently underway. Also, aerosolization of plasma-purified  $\alpha_1$ -antitrypsin and recombinant  $\alpha_1$ -antitrypsin is effective at delivery to both the alveolar surface and alveolar interstitium, but whether the amount of  $\alpha_1$ -antitrypsin is of sufficient quantity for clinical efficacy has not yet been evaluated<sup>125, 126</sup>.

Other treatments for A1ATD-associated lung disease include therapeutics commonly used for the management of COPD, including short-acting and long-acting beta adrenergic receptor agonists, muscarinic receptor antagonists (anticholinergics), in addition to influenza and pneumonia vaccination

Currently, there is no specific treatment for  $\alpha_1$ -antitrypsin liver disease and treatments for progressive liver injury are primarily supportive and focus on preventing malnutrition, rickets, and managing the complications of portal hypertension such as ascites or variceal bleeding. Patients with A1ATD-associated cirrhosis can remain stable with compensated disease and minimal signs and symptoms for years <sup>127</sup>. In these patients, the detection of cirrhosis with portal hypertension is critical, so the patient can be cautioned against the development of splenic injury from playing contact sports, advised to abstain from alcohol, undergo surveillance for variceal bleeding, and cautioned to avoid the use of NSAIDs. Consumption of NSAIDs in patients with portal hypertension can result in life-threatening bleeding, even in well-compensated patients. There are no data regarding alcohol consumption in individuals with PI\*ZZ who have no evidence of liver injury. AASLD guidelines for adults with hepatitis C without evidence of liver injury suggest that up to three alcoholic drinks per week may be safe.

For life-threatening, progressive liver failure or uncompensated cirrhosis, liver transplantation is the only therapeutic option. In the United States, patients at the highest need for liver transplant are classified by empirically derived severity scores for both children and adults, which are correlated with an increasing risk of mortality without transplant. The early evaluation at a transplant centre is recommended for patients with signs or symptoms of deteriorating liver function, although in the United States neither early addition to the transplant wait list or the time spent on the list influence the severity scores. In other countries, wait lists and liver transplantation is highly variable and is often influenced by time to referral, waiting and centre-specific factors. Many transplant centres have reported excellent liver transplant outcomes for  $\alpha_1$ -antitrypsin deficiency and outcomes are often better in  $\alpha_1$ -antitrypsin deficient patients, compared to those with other liver diseases; 1-year survival rates are 73% in adults and 87% children, dropping to 60% and 83%, respectively at 5 years <sup>128</sup>. In addition to cadaver-based donor transplantation, living donor liver transplants in infant (using a donated left lateral segment of the liver) and adult (using a split liver donation) patients with A1ATD are also successful, including surgeries performed using a donor liver from a patient with PI\*MZ<sup>129</sup>. Complications of liver transplantation include hepatic artery thrombosis, portal vein thrombosis, severe graft dysfunction, sepsis, intraoperative death,

lymphoproliferative disease, chronic rejection, biliary complications. Death from liver transplanatation occur primarily in the first six months , most often due to hemorrhage, infection , or graft failure ~ 5-10%.

## **[H1] Quality of life**

## **[H2] Life expectancy**

A1ATD can both shorten survival<sup>108, 115, 130-133</sup> and can compromise patients quality of life (QOL)(Table 2)<sup>134</sup>.

A1ATD is associated with considerable morbidity and mortality<sup>135</sup>. The median age at death for smokers with severe A1ATD is estimated at 40 years<sup>115</sup> and in a separate study, the cumulative survival up to age 50 was 52%<sup>132</sup>. In the largest available longitudinal study from the National Heart, Lung and Blood Institute Registry of Individuals with A1ATD<sup>108</sup>, the mortality rate was approximately 3% per year for patients with  $\alpha_1$ -antitrypsin deficiency (in which most of the subjects were either current (8%) or ex-smokers (72%)) .

FEV<sub>1</sub> is a major correlate of mortality in  $\alpha_1$ -antitrypsin deficiency and patients entering the National Heart, Lung and Blood Institute Registry with an FEV<sub>1</sub>>50% had a normal expected survival, whereas patients with a baseline FEV<sub>1</sub><15% had a 3-year mortality rate of 36%<sup>133</sup>. In a Danish Registry of 347 patients, the median survival of patients with a FEV<sub>1</sub><25% was 6.3 years but increased for patients with FEV<sub>1</sub>>25% (10.5 years) and FEV<sub>1</sub>>50% (14.2 years)<sup>130</sup>. FEV<sub>1</sub><sup>136</sup> and thoracic CT densitometry<sup>137</sup>, are both important predictors of patient survival, with a more rapid deterioration associated with the patient being a current smoker, aged between 30–44 years, of male sex, a predicted FEV<sub>1</sub> between 35–60% and a history of asthmatic features, chronic bronchitis and episodes of pneumonia<sup>136, 138</sup>.

COPD is less prevalent and patient survival is longer in never-smokers with A1ATD. For example, the median age at death of never smokers with COPD and A1ATD has been estimated as 65 years, versus 40 years for patients with COPD and A1ATD who smoked<sup>115</sup>. Also, never-smoking individuals with PI\*ZZ who were asymptomatic had a normal lifespan (odds ratio for death = 0.7 compared with age- and

gender-matched peers)<sup>131</sup>. Other factors contributing to prognosis of patients with A1ATD include the initial reason that prompted diagnosis of A1ATD, for example, the standardized mortality ratio for patients with A1ATD is highest (5.0) in patients who are diagnosed following the development of liver symptoms<sup>131</sup>.

The most frequent cause of death among patients with A1ATD is COPD or sequelae<sup>133</sup>, including malignancy (not HCC), diverticulitis, sepsis and/or infection and trauma. In the NHLBI Registry, emphysema accounted for 72% of deaths and cirrhosis for 10% in all patients with A1ATD<sup>133</sup>, whereas among PI\*ZZ never smokers, emphysema accounted for fewer deaths (45%) but liver disease for more (28%)<sup>131</sup>.

## [H2] Associated complications and quality of life

A1ATD also contributes to substantial morbidity and impaired QOL. As with patients with COPD, patients with A1ATD-associated COPD experience depression (25%), clinically relevant anxiety (36%), dyspnoea and impaired health-related QOL<sup>139</sup>. The number of patients with anxiety and depression was similar among patients with COPD with or without A1ATD, but dyspnoea was more common in patients with A1ATD (assessed using the Modified Medical Research Council Dyspnea Scale) and so was their health-related QOL (based on the St. George's Respiratory Questionnaire (SGRQ)). In a series of 1062 individuals with severe A1ATD<sup>134</sup>, those >59 years of age experienced fewer exacerbations and had better QOL scores (SGRQ and the Short Form (36) Health Survey) than younger individuals.

Healthcare-related QOL improved in patients with A1ATD receiving augmentation therapy following participation in a disease management program, consisting of directed patient self-education (with a comprehensive reference guide describing COPD and A1ATD) and organized supervision (monthly telephone conversations with A1ATD program coordinators, supervising participants' understanding of their long-term treatment plans)<sup>140</sup>. Participation in disease management programs was also associated with a 1-year improvement in medication adherence, enhanced compliance with supplemental oxygen and reductions in the use of some healthcare resources (though not overall hospitalization rates)<sup>140</sup>. However, although augmentation therapy alone has not been shown to improve health-related

quality of life measures, despite slowing the progression of emphysema and exacerbations<sup>141, 142</sup>.

## [H1] Outlook

The reasons why the clinical presentation of disease in individuals with Pi\*ZZ is so variable, remain unclear, but this might, in part, be due to the contributions of genetic modifiers, such as *MAN1B1*<sup>73</sup>. The use of iPSCs from patients with A1ATD and severe liver disease<sup>143, 144</sup>, which show delayed clearance of Z-type  $\alpha_1$ -antitrypsin and have a more prominent accumulation of inclusions when differentiated into hepatocytes, combined with whole genome analysis would allow characterization of these differences and is likely to clarify the effect of genetic modifiers and help to identify patients more likely to develop liver disease.

## [H2] Augmentation therapy

Augmentation therapy with purified  $\alpha_1$ -antitrypsin is not universally accepted for the prevention of emphysema in patients with A1ATD, although recent trials using surrogate endpoints, such as CT lung density measurements, for lung protection have been encouraging<sup>141, 142, 145</sup>. On balance, most evidence supports the efficacy of augmentation therapy in slowing the progression of emphysema in patients with A1ATD. Augmentation therapy has some downsides, namely, that the treatment is expensive and requires repeated, lifelong, intravenous infusions. Moreover, the estimate of the level of plasma  $\alpha_1$ -antitrypsin required to protect against emphysema (11 $\mu$ M) is arbitrary and was based on the not fully proven hypothesis that PI\*SZ patients who do not smoke do not have an increased risk for COPD. The optimal dose of augmentation therapy has yet to be determined, as patients receiving augmentation therapy lose some of the immunomodulatory effects of  $\alpha_1$ -antitrypsin just before they receive their next infusion. Also, one study conducted in 2015 (RAPID study) suggested that higher doses of  $\alpha_1$ -antitrypsin resulted in less lung density decline, observed using CT, compared with lower doses. Also, the full mechanism of the protective action of augmentation therapy is currently unknown; if the effect of  $\alpha_1$ -antitrypsin is solely mediated by correction of the

protease-antiprotease balance, or if the beneficial effects are evident primarily due to modification of inflammation requires further research.

## **[H2] Emerging therapies**

Many new approaches are currently being examined for their use in the treatment of A1ATD. Extensive studies have been published using *in vitro* analyses of molecular structure of  $\alpha_1$ -antitrypsin and >10 different compounds have been shown to block liver injury in the PiZ mouse model (transgenic mice expressing the human Z-type  $\alpha_1$ -antitrypsin) of  $\alpha_1$ -antitrypsin liver disease, although none have been approved for human use<sup>74, 146, 147</sup>.

[H3] *SERPINA1* silencing. Therapies that target the synthesis of Z-type  $\alpha_1$ -antitrypsin, aiming to prevent the accumulation of these proteins and subsequent liver injury are currently being examined through the application of RNA inhibition technology. Preclinical data obtained from the PiZ mouse model showed the complete reversal of the liver injury following the delivery of *SERPINA1* small interfering RNAs (siRNA)<sup>148</sup>. Alnylam are undertaking a Phase I clinical trial examining the safety of siRNA-mediated inhibition of the Z-type  $\alpha_1$ -antitrypsin synthesis as a therapy for liver disease. One of the major caveats associated with the use of *SERPINA1* siRNA, is that this would prevent all  $\alpha_1$ -antitrypsin production, which could cause further lung damage. To counteract this, patients receiving  $\alpha_1$ -antitrypsin siRNA will require supplementation using, for example, transfection with the normal gene (using, for example, viral vector-mediated gene therapy) and/or augmentation therapy.

[H3] Intracellular degradation of Z-type  $\alpha_1$ -antitrypsin. Methods to accelerate the intracellular degradation of Z-type  $\alpha_1$ -antitrypsin as a treatment for liver disease have also been assessed. Data from *in vitro* and mouse model experiments have shown that enhanced macroautophagy can reduce the burden of Z-type  $\alpha_1$ -antitrypsin in the liver and also reduce liver injury<sup>74, 146, 147</sup>. Drugs that enhance autophagy, such as sirolimus (also known as rapamycin), carbamazepine, and the bile acid 24-norursodeoxycholic acid



(norUDCA), in addition to a genetic approach used to augment expression of key autophagy regulators, have all been shown to reduce Z-type  $\alpha_1$ -antitrypsin accumulation in hepatocytes and to reduce liver cell injury in a mouse model system<sup>74, 146</sup>. However, excessively high doses of all of these agents were required to show an effect. A phase II clinical trial assessing low dose carbamazepine in patients with PI\*ZZ-associated cirrhosis is currently underway, although results to date have been inconclusive (NCT01379469).

[H3] Improving  $\alpha_1$ -antitrypsin folding. Other studies have focused on the use of chemical chaperone approaches to improve correct folding of  $\alpha_1$ -antitrypsin and to augment the secretion of Z  $\alpha_1$ -antitrypsin instead of retention in the liver (Figure 5). These approaches might be able to treat both the lung and the liver, however; the primary barrier to this is the large volume  $\alpha_1$ -antitrypsin protein that is synthesized per day. If a 1:1 binding stoichiometry is needed for efficacy of these drugs a large concentration of drug would need to be delivered to hepatocyte ER, which might prove clinically difficult. Nonetheless, *in vitro* studies have shown that several compounds promote the secretion of Z  $\alpha_1$ -antitrypsin and one of these compounds, 4-phenylbutyrate (4PBA), was also effective in a mouse model<sup>149</sup>. Following on from these studies, a pilot trial was conducted in patients, which showed no effect on  $\alpha_1$ -antitrypsin secretion<sup>150</sup>. Results from this trial are likely explained due to the inability of drug levels to reach the therapeutic range in patients, as those documented in the mouse model<sup>150</sup>.

Protein folding systems found within different cellular compartments are highly intertwined<sup>151</sup> and data from some studies suggest that targeting maladaptive protein folding responses in the cytosol can improve the folding of substrates in the ER, including Z  $\alpha_1$ -antitrypsin<sup>152</sup>. One study has identified the histone deacetylase 7 inhibitor suberoylanilide hydroxamic acid as molecule that can restore Z  $\alpha_1$ -antitrypsin secretion from epithelial cells<sup>153</sup>.

[H3] Inhibiting  $\alpha_1$ -antitrypsin polymerization. Strategies designed *in silico* or using cell free systems for the therapeutic disruption of Z-type  $\alpha_1$ -antitrypsin polymerization,

(likely an event separate to the protein retention signal), have also been examined<sup>147, 154</sup>. These largely peptide-based approaches aim to modulate the conformational behaviour of  $\alpha_1$ -antitrypsin by targeting it directly to fold correctly, stabilize functional conformers and limit the population of polymerogenic intermediates<sup>155-161</sup>. However, many of these compounds had an aberrant effect following examination *in vitro* and there have been difficulties into creating medicinal molecules for use in animal models. Moreover, compounds sharing a similar structure to the reactive loop of  $\alpha_1$ -antitrypsin (reactive loop analogues) can generate complexes with  $\alpha_1$ -antitrypsin that do not show antiprotease activity. Nonetheless, these molecules could still have potential for the treatment of  $\alpha_1$ -antitrypsin accumulation in hepatocytes.

[H3] M-type  $\alpha_1$ -antitrypsin synthesis. Finally, several studies that have progressed to evaluation in clinical trials, have evaluated strategies to synthesize M-type  $\alpha_1$ -antitrypsin in tissues outside the liver, which might then lead to an increase in serum levels to protect patients from lung, but which would not change the risk of liver injury<sup>162, 163</sup>. However, to date, these studies have only been able to generate less than 5% of the serum M-type  $\alpha_1$ -antitrypsin level thought to be needed for a therapeutic benefit in humans using an adeno-associated vector carrying *SERPINA1*<sup>163</sup>. Various gene therapy approaches have been designed to increase the levels of circulating  $\alpha_1$ -antitrypsin with one having reached Phase II testing<sup>163-168</sup>. Two of these approaches involve haematopoietic stem cell therapy, coupled with delivery of lentiviral vector containing *SERPINA1*<sup>169, 170</sup> or intrapleural administration of a replication-deficient adeno-associated viral vector containing the *SERPINA1* transgene<sup>171</sup>.

Other future potential treatments include the use of induced pluripotent stem cells<sup>143, 172, 173</sup> generated from skin fibroblasts harvested from individuals with PI\*ZZ, then reprogrammed to form hepatocyte-like cells that still possess the A1ATD phenotype<sup>143</sup>. Using the CRISPR-CAS9 system to correct the Z mutation in these cells<sup>172</sup> could generate PI\*MM cells, that share the characteristics of those found in healthy individuals. Theoretically, these cells could be used for autologous grafting without the risk of immune rejection. *In vitro* reports using this technique are still limited and no clinical

trials have been commenced, however; this approach is still promising and might be a long term answer to the treatment of both lung and liver disease manifestations of this  $\alpha_1$ -antitrypsin deficiency.

## [H2] Other disorders

$\alpha_1$ -antitrypsin is one member of a large family of serine protease inhibitors, known as serpins. Other members of this protease family are mutated in human diseases and so it is likely that data obtained from the study of  $\alpha_1$ -antitrypsin will have wider biomedical application. For example, mutant forms of the neuron-specific serpin (neuroserpin) also undergoes polymerization and formation of inclusion bodies in the same way as  $\alpha_1$ -antitrypsin, but accumulation of mutant neuroserpin leads to neurodegeneration and early onset dementia<sup>174</sup>. The development of therapeutics that prevent the polymerization of  $\alpha_1$ -antitrypsin will also rapidly lead to the development of therapies for other serpinopathies that are primarily associated with accumulation of polymers. Similarly, the development of small molecules designed to mimic the anti-inflammatory effects of  $\alpha_1$ -antitrypsin would be applicable for use in other disorders, since  $\alpha_1$ -antitrypsin augmentation therapy has been shown to be beneficial in other diseases, including cystic fibrosis<sup>175, 176</sup>.

Finally, the mechanism by which accumulation of polymers of  $\alpha_1$ -antitrypsin in the lumen of the ER can trigger downstream signalling is unknown, but it has been proposed that the ER overload seen in this condition, might also mediate cellular responses to enveloped viruses. The study of  $\alpha_1$ -antitrypsin is highly important and could improve knowledge of other more prevalent conditions<sup>177, 178</sup>.

## **Acknowledgements**

Funding for research in C.M.G's group is gratefully acknowledged from The Health Research Board, Science Foundation Ireland, The Irish Research Council, The National Children's Research Centre, The European Respiratory Society, Horizon 2020, Cystic Fibrosis Foundation Therapeutics, and the Alpha One Foundation USA. The authors would like to thank Dr James Irving, UCL, UK for preparing Figure 2.

## **Author contributions**

Introduction (C.M.G.); Epidemiology (C.M.G. and J.K.S.); Mechanisms/pathophysiology (C.M.G., D.A.L., I.F., S.J.M., and J.H.T.); Diagnosis, screening and prevention (C.M.G., M.L.B., J.K.S. and N.G.M.); Management (C.M.G., J.H.T., and N.G.M.); Quality of life (C.M.G. and J.K.S.); Outlook (C.M.G., S.J.M., and N.G.M.); Overview of Primer (C.M.G.).

## **Competing interests**

C.M.G. has received research grants from the Alpha-1 Foundation and received an honorarium for educational materials from Vertex Pharmaceuticals. J.H.T. has served as a consultant for Alnylam Pharmaceuticals, Arrowhead Research, Proteostasis Therapeutics, Isis Pharmaceuticals (now Ionis Pharmaceuticals), Editas Medicine, Genkyotex, GLG Pharma, INSERM, Intellia Therapeutics, Retrophin, RxCelerate, Velgene. He also received honoraria for speaking from the Alpha-1 Foundation and the Cystic Fibrosis Foundation and research grants or support from the Alpha-1 Foundation, National Institutes of Health USA, Alnylam Pharmaceuticals, Arrowhead Research and the Cardinal Glennon Children's Foundation. M.L.B. has received research support for clinical trials from Baxalta, Kamada and Grifols, is an owner of GeneAidyx, a genetic diagnostic company, and holds patents for AAT gene therapy and compounds to modify AAT secretion. D.A.L. has received research funding from GlaxoSmithKline to develop small molecule therapies for antitrypsin deficiency. He was also chair of the GlaxoSmithKline Respiratory Therapy Area Board between 2012–2015. J.K.S. has served as a consultant to Kamada, Grifols, Arrowhead Research, CSL Behring, Baxalta, Pfizer and Boehringer-Ingelheim. He is a member of the board of directors of the Alpha-

1 Foundation, and the Medical and Scientific Advisory Council for both the COPD Foundation and the Alpha-1 Foundation. N.G.M. has served as a consultant for Chiesi and Bayer and received honoraria for speaking from Chiesi, Grifols and CSL Behring. He also received grants or research support from Chiesi, Grifols, Vertex, and the Alpha-1 Foundation. S.J.M. and I.F. declare no competing interests.

### Figure legends

Figure 1 | Clinical manifestations of PI\*ZZ. About 15% of infants with PI\*ZZ genotype have or develop clinical relevant liver disease. The clinical symptoms of jaundice typically resolve by the second year of life but can progress to cirrhosis in 15% of children who had jaundice at birth<sup>5, 179</sup>. The risk of death from liver disease in children with PI\*ZZ is between 2-3%<sup>180, 181</sup>. Serum aminotransferases and bilirubin levels (markers for liver dysfunction) are raised in 70% and 11% of infants, respectively, in their first year of life but the levels typically go down. Raised aminotransferases remain abnormal in 15% of patients by 12 years of age and serum bilirubin levels fall to within the normal range by 6 months of age. Some adults with Z-type A1ATD have slowly progressive hepatic damage that is asymptomatic and is only apparent as a minor degree of portal fibrosis. A1ATD-associated cirrhosis can present in adults of any age. 13% of patients with PI\*ZZ die from liver disease<sup>10</sup>. Lung dysfunction develops between the third and fourth decade of life. Emphysema associated with Z-type A1ATD is typically panlobular and affects the bases of the lungs. Patients initially present with breathlessness and cor pulmonale (enlargement of the right side of the heart owing to lung disease) and polycythaemia (increased red blood cell levels in the blood can occur late in the disease<sup>99</sup>. PI\*ZZ A1ATD is also associated with an increased prevalence of asthma<sup>182</sup>, panniculitis<sup>183</sup> and granulomatosis with polyangiitis<sup>184</sup>. The underlying disease mechanisms are currently unknown but pro-inflammatory polymers of Z  $\alpha_1$ -antitrypsin and deficiency of M-type  $\alpha_1$  antitrypsin might contribute to both granulomatosis with polyangiitis and panniculitis.

Figure 2 | *SERPINA* and  $\alpha_1$ -antitrypsin. A | *SERPINA* gene, which encodes  $\alpha_1$ -antitrypsin, is located on the long arm of chromosome 14 at 14q32.1. The gene consists of four coding exons (II, III, IV, and V), three untranslated exons (Ia, Ib, and Ic) and six introns. Distinct promoters and transcription start-sites in the 5'-untranslated region (5'-UTR) have been identified for hepatocytes and extra-hepatic tissues such as monocytes/macrophages and the cornea<sup>21</sup>. The hepatocyte *SERPINA* promoter is located within exon 1C, upstream of the hepatocyte transcription start site<sup>21, 185</sup>. Alternative promoter regions are located upstream of exon 1A and before exon 1B; these control *SERPINA* expression in monocytes and macrophages<sup>21, 186</sup>. The most common A1ATD-associated mutation are depicted. B| The  $\alpha_1$ -antitrypsin protein is a 394 residue, 52 kDa glycoprotein that is synthesised by hepatocytes, lung and gut epithelial cells, neutrophils and alveolar macrophages. Structure of monomeric  $\alpha_1$ -antitrypsin with the position of key mutations shown in black  $\alpha_1$ -antitrypsin contains a 'reactive loop', which contains a neutrophil elastase binding site. Neutrophil elastase docks with, then cleaves the reactive loop of  $\alpha_1$ -antitrypsin, which causes the translocation of elastase from the upper to the lower pole of  $\alpha_1$ -antitrypsin, and irreversibly inhibits the activity of the enzyme<sup>33</sup>.

**Figure 3.** Proposed models of serpin polymerisation (key linkage motifs highlighted in black): i. Reactive centre loop-  $\beta$ -sheet A linkage, ii, linkage by a  $\beta$ -hairpin of the reactive centre loop and strand 5A and iii, linkage with strands 1C, 4B and 5B. Figure generated with PyMol by Dr James Irving, UCL, UK.

**Figure 4.** Intrapulmonary consequences of unopposed neutrophil elastase activity. Neutrophil elastase is normally inhibited by  $\alpha_1$ -antitrypsin. However, in the lung of A1ATD patients, unopposed neutrophil elastase activity can activate cell surface receptors and transcriptionally upregulates expression of classes of genes.

Figure 5 | Accumulation of misfolded  $\alpha_1$ -antitrypsin in hepatocytes. Misfolding of  $\alpha_1$ -antitrypsin leads to  $\alpha_1$ -antitrypsin accumulation in the ER, which either gets degraded via the ERAD pathway or macroautophagy. When the capacity of these processes is exceeded,  $\alpha_1$ -antitrypsin polymers accumulates which, depending on the level of

accumulation, results in oxidative stress and cell death, or proliferation. The final outcome is liver failure. Potential interventions are depicted in red boxes.

**Figure 6.** Fates of  $\alpha_1$ -antitrypsin within the endoplasmic reticulum.

The nascent  $\alpha_1$ -antitrypsin protein is translated and enters the endoplasmic reticulum (ER) where it undergoes N-linked glycosylation. Glucose residues are trimmed from  $\alpha_1$ -antitrypsin by the action of glucosidases I (GS1) and glucosidases II (GS2), which promotes interaction of the nascent protein with the lectin chaperones calnexin (CNX) and calreticulin (CRT). This allows the folding of the protein by protein disulphide-isomerase A3 (ERp57), following which, it is deglycosylated by GS2<sup>248</sup>. If folding of the  $\alpha_1$ -antitrypsin protein is correct, it is exported from the ER and trafficked to the Golgi apparatus. Conversely, misfolded  $\alpha_1$ -antitrypsin protein (for example, NHK or Z isoforms) is reglycosylated by UDP-glucose:glycoprotein glucosyltransferase 1 (UGGT1), which allows the reinteraction of misfolded  $\alpha_1$ -antitrypsin with CNX and CRT and refolding by ERp57. This cycle can persist, but misfolded  $\alpha_1$ -antitrypsin eventually undergoes demannosylation by ER  $\alpha$ -mannosidase I (ERManI and EDEM). Further demannosylation leads to the interaction of  $\alpha_1$ -antitrypsin with protein OS-9 (OS-9) and endoplasmic (GRP94) and delivery to the E3 ubiquitin-protein ligase synoviolin (HRD1) complex (containing E3 ubiquitin-protein ligase AMFR (gp78)) for ER associated degradation (ERAD)<sup>116</sup>. Misfolded  $\alpha_1$ -antitrypsin (for example the NHK isoform) can also accumulate in the ER and is speculated to sequester BiP away from the ER stress sensors eukaryotic translation initiation factor 2- $\alpha$  kinase 3 (PERK), cyclic-AMP-dependent transcription factor ATF-6 $\alpha$  (ATF6) and serine/threonine-protein kinase/endoribonuclease IRE1 (IRE1), leading to activation of the unfolded protein response (UPR) and ER stress. By contrast, accumulation of Z  $\alpha_1$ -antitrypsin results in the formation of ordered polymers that lead to the activation of the ER overload response (EOR) and upregulation of proinflammatory cytokines. The underlying mechanisms of polymer-mediated activation of EOR are poorly understood, but appear to require calcium release of calcium from the ER lumen, NF- $\kappa$ B signalling and a proinflammatory

response. Under some circumstances, polymers of  $\alpha_1$ -antitrypsin can be degraded by macroautophagy.

**Figure 7.** One diagnostic algorithm for A1ATD. A1ATD diagnosis involves quantification of  $\alpha_1$ -antitrypsin levels, genotyping of *SERPINA1* and PI typing.



**Table 1.**  $\alpha_1$ -antitrypsin augmentation therapy observational studies and clinical trials

<b>Clinical trial design</b>	<b>Lead author or group name</b>	<b>Year</b>	<b>Main outcome</b>	<b>Duration of follow up</b>	<b>Ref .</b>
Multicenter, randomised	Chapman (RAPID Study)	2015	Slower rate of lung tissue loss assessed using CT	2-4 years	<sup>142</sup>
Multicentre, randomised, placebo-controlled, double-blind	Dirksen EXACTLE Study)	2009	Trend towards slower rate of lung tissue loss assessed using CT	2-2.5 years	<sup>141</sup>
	Dirksen	1999	Trend towards slower rate of lung tissue loss assessed using CT and increased mortality	3 years	<sup>145</sup>
Observational	Seersholm	1997	Reduction in FEV <sub>1</sub> decline in cohort with FEV <sub>1</sub> 31-65%	>1 year	<sup>187</sup>
Observational	NHLBI Registry	1998	Slower rate of FEV <sub>1</sub> decline in cohort with a baseline FEV <sub>1</sub> between 35-49%	7.2 years	<sup>108</sup>
Observational	Lieberman	2000	Reductions in the incidence of lung infections	1-10 years	<sup>188</sup>
Observational	Wencker	2001	Slower rate of FEV <sub>1</sub> decline	>12 months	<sup>189</sup>
Observational	Tonelli	2009	Slower rate of FEV <sub>1</sub> decline	3.5 years	<sup>190</sup>

**Table 2.** Factors affecting symptoms and QOL in  $\alpha_1$ -antitrypsin deficient patients with COPD<sup>139</sup>

Symptom	Comment
Depression	Decreased in those in a stable relationship rather than single
Anxiety	Increased in those who are younger and less educated
Dyspnoea	Worse if the patient is single compared with patients who were married or in a long-term relationship, and compared with non- $\alpha_1$ -antitrypsin deficient patients with COPD
Impaired QOL	Poorer compared to non- $\alpha_1$ -antitrypsin deficient patients with COPD, but less severe above 59 years of age

## References

1. Laurell CB, E.S. The electrophoretic alpha-globulin pattern of serum in alpha1-antitrypsin deficiency. *Scand J Clin Lab Invest.* **15**, 132-140 (1963).
2. Stoller, J.K. & Brantly, M. The challenge of detecting alpha-1 antitrypsin deficiency. *COPD* **10 Suppl 1**, 26-34 (2013).
3. Stoller, J.K., Smith, P., Yang, P. & Spray, J. Physical and social impact of alpha 1-antitrypsin deficiency: results of a survey. *Cleve Clin J Med* **61**, 461-7 (1994).
4. de Serres, F.J., Blanco, I. & Fernandez-Bustillo, E. PI S and PI Z alpha-1 antitrypsin deficiency worldwide. A review of existing genetic epidemiological data. *Monaldi Arch Chest Dis* **67**, 184-208 (2007).
5. Sveger, T. Liver disease in alpha<sub>1</sub>-antitrypsin deficiency detected by screening of 200,000 infants. *N. Engl. J. Med.* **294**, 1316-1321 (1976).
6. Silverman, E.K. et al. Alpha-1-antitrypsin deficiency. High prevalence in the St. Louis area determined by direct population screening. *Am Rev Respir Dis* **140**, 961-6 (1989).
7. Thun, G.A. et al. Causal and synthetic associations of variants in the SERPINA gene cluster with alpha1-antitrypsin serum levels. *PLoS Genet* **9**, e1003585 (2013).
8. O'Brien, M.E. et al. The impact of smoke exposure on the clinical phenotype of alpha-1 antitrypsin deficiency in Ireland: exploiting a national registry to understand a rare disease. *COPD* **12 Suppl 1**, 2-9 (2015).
9. Tanash, H.A., Nystedt-Düzakin, M., Montero, L.C., Sveger, T. & Piitulainen, E. The Swedish  $\alpha$ 1-Antitrypsin Screening Study: Health status and lung and liver function at age 34. *Ann Am Thorac Soc.* **12**, 807-812 (2015).
10. Piitulainen, E. & Tanash, H.A. The Clinical Profile of Subjects Included in the Swedish National Register on Individuals with Severe Alpha 1-Antitrypsin deficiency. *COPD* **12 Suppl 1**, 36-41 (2015).
11. McAloon, C.J., Wood, A.M., Gough, S.C. & Stockley, R.A. Matrix metalloprotease polymorphisms are associated with gas transfer in alpha 1 antitrypsin deficiency. *Thorax* **64**, 23-30 (2009).
12. Wood, A.M. et al. The TNFalpha gene relates to clinical phenotype in alpha-1-antitrypsin deficiency. *Respir Res* **9**, 52 (2008).
13. Demeo, D.L. et al. IL10 polymorphisms are associated with airflow obstruction in severe alpha1-antitrypsin deficiency. *Am J Respir Cell Mol Biol* **38**, 114-20 (2008).
14. Kim, W.J. et al. Association of IREB2 and CHRNA3 polymorphisms with airflow obstruction in severe alpha-1 antitrypsin deficiency. *Respir Res* **13**, 16 (2012).
15. Alam, S. et al. Z alpha1-antitrypsin confers a proinflammatory phenotype that contributes to chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **189**, 909-31 (2014).
16. Wood, A.M., Harrison, R.M., Semple, S., Ayres, J.G. & Stockley, R.A. Outdoor air pollution is associated with disease severity in alpha1-antitrypsin deficiency. *Eur Respir J* **34**, 346-53 (2009).

17. Wood, A.M., Harrison, R.M., Semple, S., Ayres, J.G. & Stockley, R.A. Outdoor air pollution is associated with rapid decline of lung function in alpha-1-antitrypsin deficiency. *Occup Environ Med* **67**, 556-61 (2010).
18. Mehta, A.J. et al. Interactions between SERPINA1 PiMZ genotype, occupational exposure and lung function decline. *Occup Environ Med* **71**, 234-40 (2014).
19. Molloy, K. et al. Clarification of the risk of chronic obstructive pulmonary disease in alpha1-antitrypsin deficiency PiMZ heterozygotes. *Am J Respir Crit Care Med* **189**, 419-27 (2014).
20. Parmar, J.S. et al. Polymers of alpha(1)-antitrypsin are chemotactic for human neutrophils: a new paradigm for the pathogenesis of emphysema. *Am J Respir Cell Mol Biol* **26**, 723-30 (2002).
21. Kalsheker, N., Morley, S. & Morgan, K. Gene regulation of the serine proteinase inhibitors alpha1-antitrypsin and alpha1-antichymotrypsin. *Biochem Soc Trans* **30**, 93-8 (2002).
22. Hafeez, W., Ciliberto, G. & Perlmutter, D.H. Constitutive and modulated expression of the human alpha 1 antitrypsin gene. Different transcriptional initiation sites used in three different cell types. *J Clin Invest* **89**, 1214-22 (1992).
23. Knoell, D.L., Ralston, D.R., Coulter, K.R. & Wewers, M.D. Alpha 1-antitrypsin and protease complexation is induced by lipopolysaccharide, interleukin-1beta, and tumor necrosis factor-alpha in monocytes. *Am J Respir Crit Care Med* **157**, 246-55 (1998).
24. Cichy, J., Potempa, J. & Travis, J. Biosynthesis of alpha1-proteinase inhibitor by human lung-derived epithelial cells. *J Biol Chem* **272**, 8250-5 (1997).
25. Sallenave, J.M., Tremblay, G.M., Gauldie, J. & Richards, C.D. Oncostatin M, but not interleukin-6 or leukemia inhibitory factor, stimulates expression of alpha1-proteinase inhibitor in A549 human alveolar epithelial cells. *J Interferon Cytokine Res* **17**, 337-46 (1997).
26. Cichy, J., Rose-John, S. & Travis, J. Oncostatin M, leukaemia-inhibitory factor and interleukin 6 trigger different effects on alpha1-proteinase inhibitor synthesis in human lung-derived epithelial cells. *Biochem J* **329** ( Pt 2), 335-9 (1998).
27. Kulig, P. & Cichy, J. Acute phase mediator oncostatin M regulates affinity of alpha1-protease inhibitor for concanavalin A in hepatoma-derived but not lung-derived epithelial cells. *Cytokine* **30**, 269-74 (2005).
28. Bosco, D. et al. Expression and secretion of alpha1-proteinase inhibitor are regulated by proinflammatory cytokines in human pancreatic islet cells. *Diabetologia* **48**, 1523-33 (2005).
29. Matamala, N. et al. Alternative transcripts of the SERPINA1 gene in alpha-1 antitrypsin deficiency. *J Transl Med* **13**, 211 (2015).
30. Gooptu, B., Dickens, J.A. & Lomas, D.A. The molecular and cellular pathology of alpha(1)-antitrypsin deficiency. *Trends Mol Med* **20**, 116-27 (2014).
31. Silva, D. et al. Alpha-1-antitrypsin (SERPINA1) mutation spectrum: Three novel variants and haplotype characterization of rare deficiency alleles identified in Portugal. *Respiratory Medicine* **116**, 8-18 (2016).
32. Ferrarotti, I. et al. Identification and characterisation of eight novel SERPINA1 Null mutations. *Orphanet J Rare Dis* **9**, 172 (2014).

33. Huntington, J.A., Read, R.J. & Carrell, R.W. Structure of a serpin-protease complex shows inhibition by deformation. *Nature* **407**, 923-926 (2000).
34. Lomas, D.A., Evans, D.L., Finch, J.T. & Carrell, R.W. The mechanism of Z  $\alpha_1$ -antitrypsin accumulation in the liver. *Nature* **357**, 605-607 (1992).
35. Miranda, E. et al. A novel monoclonal antibody to characterise pathogenic polymers in liver disease associated with  $\alpha_1$ -antitrypsin deficiency. *Hepatology* **52**, 1078-1088 (2010).
36. Lomas, D.A., Finch, J.T., Seyama, K., Nukiwa, T. & Carrell, R.W.  $\alpha_1$ -antitrypsin S<sub>iiyama</sub> (Ser<sup>53</sup>ØPhe); further evidence for intracellular loop-sheet polymerisation. *J. Biol. Chem.* **268**, 15333-15335 (1993).
37. Lomas, D.A. et al. Alpha<sub>1</sub>-antitrypsin Mmalton (<sup>52</sup>Phe deleted) forms loop-sheet polymers *in vivo*: evidence for the C sheet mechanism of polymerisation. *J. Biol. Chem.* **270**, 16864-16870 (1995).
38. Ordonez, A. & Marciniak, S.J. in *The Serpin Family: Proteins with Multiple Functions in Health and Disease* (ed. Geiger, M.e.a.) (2015).
39. Irving, J.A., Haq, I., Dickens, J.A., Faull, S.V. & Lomas, D.A. Altered native stability is the dominant basis for susceptibility of alpha1-antitrypsin mutants to polymerization. *Biochem J* **460**, 103-15 (2014).
40. Yamasaki, M., Li, W., Johnson, D.J. & Huntington, J.A. Crystal structure of a stable dimer reveals the molecular basis of serpin polymerization. *Nature* **455**, 1255-1258 (2008).
41. Yamasaki, M., Sendall, T.J., Pearce, M.C., Whisstock, J.C. & Huntington, J.A. Molecular basis of  $\alpha_1$ -antitrypsin deficiency revealed by the structure of a domain-swapped trimer. *EMBO Rep.* **12**, 1011-1017 (2011).
42. Krishnan, B. & Gierasch, L.M. Dynamic local unfolding in the serpin  $\alpha$ -1 antitrypsin provides a mechanism for loop insertion and polymerization. *Nat Struct Mol Biol* **18**, 222-226 (2011).
43. Behrens, M.A. et al. The shapes of Z- $\alpha_1$ -antitrypsin polymers in solution support the C-terminal domain-swap mechanism of polymerization. *Biophysical J.* **107**, 1905-1912 (2014).
44. Weldon, S. et al. Decreased levels of secretory leucoprotease inhibitor in the Pseudomonas-infected cystic fibrosis lung are due to neutrophil elastase degradation. *J Immunol* **183**, 8148-56 (2009).
45. Nemoto, E. et al. Cleavage of CD14 on human gingival fibroblasts cocultured with activated neutrophils is mediated by human leukocyte elastase resulting in down-regulation of lipopolysaccharide-induced IL-8 production. *J Immunol* **165**, 5807-13 (2000).
46. Hartl, D. et al. Cleavage of CXCR1 on neutrophils disables bacterial killing in cystic fibrosis lung disease. *Nat Med* **13**, 1423-30 (2007).
47. Bergin, D.A. et al. alpha-1 Antitrypsin regulates human neutrophil chemotaxis induced by soluble immune complexes and IL-8. *J Clin Invest* **120**, 4236-50 (2010).
48. Bergin, D.A. et al. The circulating proteinase inhibitor alpha-1 antitrypsin regulates neutrophil degranulation and autoimmunity. *Sci Transl Med* **6**, 217ra1 (2014).

49. Yasutake, A. & Powers, J.C. Reactivity of human leukocyte elastase and porcine pancreatic elastase toward peptide 4-nitroanilides containing model desmosine residues. Evidence that human leukocyte elastase is selective for cross-linked regions of elastin. *Biochemistry* **20**, 3675-9 (1981).
50. Kafienah, W., Buttle, D.J., Burnett, D. & Hollander, A.P. Cleavage of native type I collagen by human neutrophil elastase. *Biochem J* **330** ( Pt 2), 897-902 (1998).
51. McDonald, J.A. & Kelley, D.G. Degradation of fibronectin by human leukocyte elastase. Release of biologically active fragments. *J Biol Chem* **255**, 8848-58 (1980).
52. Malemud, C.J. & Janoff, A. Identification of neutral proteases in human neutrophil granules that degrade articular cartilage proteoglycan. *Arthritis Rheum* **18**, 361-8 (1975).
53. Geraghty, P. et al. Neutrophil elastase up-regulates cathepsin B and matrix metalloprotease-2 expression. *J Immunol* **178**, 5871-8 (2007).
54. Shao, M.X. & Nadel, J.A. Dual oxidase 1-dependent MUC5AC mucin expression in cultured human airway epithelial cells. *Proc Natl Acad Sci U S A* **102**, 767-72 (2005).
55. Shao, M.X. & Nadel, J.A. Neutrophil elastase induces MUC5AC mucin production in human airway epithelial cells via a cascade involving protein kinase C, reactive oxygen species, and TNF-alpha-converting enzyme. *J Immunol* **175**, 4009-16 (2005).
56. Bergin, D.A. et al. Activation of the epidermal growth factor receptor (EGFR) by a novel metalloprotease pathway. *J Biol Chem* **283**, 31736-44 (2008).
57. Okada, Y. et al. Inactivation of tissue inhibitor of metalloproteinases by neutrophil elastase and other serine proteinases. *FEBS Lett* **229**, 157-60 (1988).
58. Guyot, N. et al. Elafin, an elastase-specific inhibitor, is cleaved by its cognate enzyme neutrophil elastase in sputum from individuals with cystic fibrosis. *J Biol Chem* **283**, 32377-85 (2008).
59. Abrahamson, M. et al. Human cystatin C. role of the N-terminal segment in the inhibition of human cysteine proteinases and in its inactivation by leucocyte elastase. *Biochem J* **273** ( Pt 3), 621-6 (1991).
60. Greene, C.M., Hassan, T., Molloy, K. & McElvaney, N.G. The role of proteases, endoplasmic reticulum stress and SERPINA1 heterozygosity in lung disease and alpha-1 anti-trypsin deficiency. *Expert Rev Respir Med* **5**, 395-411 (2011).
61. Petrache, I. et al. alpha-1 antitrypsin inhibits caspase-3 activity, preventing lung endothelial cell apoptosis. *Am J Pathol* **169**, 1155-66 (2006).
62. O'Dwyer, C.A. et al. The BLT1 Inhibitory Function of alpha-1 Antitrypsin Augmentation Therapy Disrupts Leukotriene B4 Neutrophil Signaling. *J Immunol* **195**, 3628-41 (2015).
63. Hurley, K. et al. Alpha-1 antitrypsin augmentation therapy corrects accelerated neutrophil apoptosis in deficient individuals. *J Immunol* **193**, 3978-91 (2014).
64. Geraghty, P. et al. alpha1-Antitrypsin activates protein phosphatase 2A to counter lung inflammatory responses. *Am J Respir Crit Care Med* **190**, 1229-42 (2014).
65. Lindblad, D., Blomenkamp, K. & Teckman, J. Alpha-1-antitrypsin mutant Z protein content in individual hepatocytes correlates with cell death in a mouse model. *Hepatology* **46**, 1228-35 (2007).

66. Kroeger, H. et al. Endoplasmic reticulum-associated degradation (ERAD) and autophagy cooperate to degrade polymerogenic mutant serpins. *J Biol Chem* **284**, 22793-802 (2009).
67. Teckman, J.H. et al. The proteasome participates in degradation of mutant alpha 1-antitrypsin Z in the endoplasmic reticulum of hepatoma-derived hepatocytes. *J Biol Chem* **276**, 44865-72 (2001).
68. Kruse, K.B., Brodsky, J.L. & McCracken, A.A. Characterization of an ERAD gene as VPS30/ATG6 reveals two alternative and functionally distinct protein quality control pathways: one for soluble Z variant of human alpha-1 proteinase inhibitor (A1PiZ) and another for aggregates of A1PiZ. *Mol Biol Cell* **17**, 203-12 (2006).
69. Ekeowa, U.I. et al. Defining the mechanism of polymerization in the serpinopathies. *Proc. Natl. Acad. Sci. USA* **107**, 17146-17151 (2010).
70. Hosokawa, N. et al. Enhancement of endoplasmic reticulum (ER) degradation of misfolded Null Hong Kong alpha1-antitrypsin by human ER mannosidase I. *J Biol Chem* **278**, 26287-94 (2003).
71. Cabral, C.M., Choudhury, P., Liu, Y. & Sifers, R.N. Processing by endoplasmic reticulum mannosidases partitions a secretion-impaired glycoprotein into distinct disposal pathways. *J Biol Chem* **275**, 25015-22 (2000).
72. Wu, Y., Swulius, M.T., Moremen, K.W. & Sifers, R.N. Elucidation of the molecular logic by which misfolded alpha 1-antitrypsin is preferentially selected for degradation. *Proc Natl Acad Sci U S A* **100**, 8229-34 (2003).
73. Pan, Z., Erkan, M., Streit, S., Friess, H. & Kleeff, J. Silencing of GRP94 expression promotes apoptosis in pancreatic cancer cells. *Int J Oncol* **35**, 823-8 (2009).
74. Hidvegi, T. et al. An autophagy-enhancing drug promotes degradation of mutant alpha1-antitrypsin Z and reduces hepatic fibrosis. *Science* **329**, 229-32 (2010).
75. Marciniak, S.J. & Lomas, D.A. Alpha1-antitrypsin deficiency and autophagy. *N Engl J Med* **363**, 1863-4 (2010).
76. Chambers, J.E. & Marciniak, S.J. Cellular mechanisms of endoplasmic reticulum stress signaling in health and disease. 2. Protein misfolding and ER stress. *Am J Physiol Cell Physiol* **307**, C657-70 (2014).
77. Ron, D. & Walter, P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol* **8**, 519-29 (2007).
78. Sifers, R.N., Brashears-Macatee, S., Kidd, V.J., Muensch, H. & Woo, S.L. A frameshift mutation results in a truncated alpha 1-antitrypsin that is retained within the rough endoplasmic reticulum. *J Biol Chem* **263**, 7330-5 (1988).
79. Brodbeck, R.M. & Brown, J.L. Secretion of alpha-1-proteinase inhibitor requires an almost full length molecule. *J Biol Chem* **267**, 294-7 (1992).
80. Faber, J.P. et al. Identification and DNA sequence analysis of 15 new alpha 1-antitrypsin variants, including two PI\*Q0 alleles and one deficient PI\*M allele. *Am J Hum Genet* **55**, 1113-21 (1994).
81. Liu, Y., Choudhury, P., Cabral, C.M. & Sifers, R.N. Intracellular disposal of incompletely folded human alpha1-antitrypsin involves release from calnexin and post-translational trimming of asparagine-linked oligosaccharides. *J Biol Chem* **272**, 7946-51 (1997).

82. Hidvegi, T., Schmidt, B.Z., Hale, P. & Perlmutter, D.H. Accumulation of mutant alpha1-antitrypsin Z in the endoplasmic reticulum activates caspases-4 and -12, NFkappaB, and BAP31 but not the unfolded protein response. *J Biol Chem* **280**, 39002-15 (2005).
83. Ordonez, A. et al. Endoplasmic reticulum polymers impair luminal protein mobility and sensitize to cellular stress in alpha1-antitrypsin deficiency. *Hepatology* **57**, 2049-60 (2013).
84. Bertolotti, A., Zhang, Y., Hendershot, L.M., Harding, H.P. & Ron, D. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. *Nat Cell Biol* **2**, 326-32 (2000).
85. Credle, J.J., Finer-Moore, J.S., Papa, F.R., Stroud, R.M. & Walter, P. On the mechanism of sensing unfolded protein in the endoplasmic reticulum. *Proc Natl Acad Sci U S A* **102**, 18773-84 (2005).
86. Graham, K.S., Le, A. & Sifers, R.N. Accumulation of the insoluble PiZ variant of human alpha 1-antitrypsin within the hepatic endoplasmic reticulum does not elevate the steady-state level of grp78/BiP. *J Biol Chem* **265**, 20463-8 (1990).
87. Lawless, M.W. et al. Activation of endoplasmic reticulum-specific stress responses associated with the conformational disease Z alpha 1-antitrypsin deficiency. *J Immunol* **172**, 5722-6 (2004).
88. Davies, M.J. et al. Neuroserpin polymers activate NF-kappaB by a calcium signaling pathway that is independent of the unfolded protein response. *J Biol Chem* **284**, 18202-9 (2009).
89. van 't Wout, E.F. et al. Increased ERK signalling promotes inflammatory signalling in primary airway epithelial cells expressing Z alpha1-antitrypsin. *Hum Mol Genet* **23**, 929-41 (2014).
90. Van't Wout, E.F. et al. Function of monocytes and monocyte-derived macrophages in alpha1-antitrypsin deficiency. *Eur Respir J* **45**, 365-76 (2015).
91. Carroll, T.P. et al. Evidence for unfolded protein response activation in monocytes from individuals with alpha-1 antitrypsin deficiency. *J Immunol* **184**, 4538-46 (2010).
92. Tan, L. et al. Circulating polymers in alpha1-antitrypsin deficiency. *Eur Respir J* **43**, 1501-4 (2014).
93. Elliott, P.R., Bilton, D. & Lomas, D.A. Lung polymers in Z alpha1-antitrypsin deficiency-related emphysema. *Am J Respir Cell Mol Biol* **18**, 670-4 (1998).
94. Mahadeva, R. et al. Polymers of Z alpha1-antitrypsin co-localize with neutrophils in emphysematous alveoli and are chemotactic in vivo. *Am J Pathol* **166**, 377-86 (2005).
95. Fra, A. et al. Polymers of Z alpha1-antitrypsin are secreted in cell models of disease. *Eur Respir J* **47**, 1005-9 (2016).
96. Blanco, I., Lipsker, D., Lara, B. & Janciauskiene, S. Neutrophilic Panniculitis Associated with Alpha-1 Antitrypsin Deficiency: an Update. *Br J Dermatol* (2015).
97. Greulich, T. et al. Alpha1-antitrypsin deficiency - diagnostic testing and disease awareness in Germany and Italy. *Respir Med* **107**, 1400-8 (2013).



98. Jain, A., McCarthy, K., Xu, M. & Stoller, J.K. Impact of a clinical decision support system in an electronic health record to enhance detection of alpha(1)-antitrypsin deficiency. *Chest* **140**, 198-204 (2011).
99. ATS/ERS. ATS/ERS statement: standards for the diagnosis and management of individuals with alpha1-antitrypsin deficiency. *Am. J. Respir. Crit. Care Med.* **168**, 818-900 (2003).
100. Brantly, M. in Alpha-1-Antitrypsin Deficiency (ed. R, C.) 211-26 (Marcel Dekker, New York, 1995).
101. McElvaney, N.G. Diagnosing alpha1-antitrypsin deficiency: how to improve the current algorithm. *Eur Respir Rev* **24**, 52-7 (2015).
102. Brantly, M. Efficient and accurate approaches to the laboratory diagnosis of alpha1-antitrypsin deficiency: The promise of early diagnosis and intervention. *Clin Chem* **52**, 2180-1 (2006).
103. Carroll, T.P. et al. The prevalence of alpha-1 antitrypsin deficiency in Ireland. *Respir Res* **12**, 91 (2011).
104. Sveger, T. & Thelin, T. Four-year-old children with alpha 1-antitrypsin deficiency. Clinical follow-up and parental attitudes towards neonatal screening. *Acta Paediatr Scand* **70**, 171-7 (1981).
105. Disease, G.I.f.C.L. (2014).
106. Alpha 1-antitrypsin deficiency: memorandum from a WHO meeting. *Bull World Health Organ* **75**, 397-415 (1997).
107. (2012).
108. Survival and FEV1 decline in individuals with severe deficiency of alpha1-antitrypsin. The Alpha-1-Antitrypsin Deficiency Registry Study Group. *Am J Respir Crit Care Med* **158**, 49-59 (1998).
109. Strange, C. et al. The United States Alpha-1 Foundation Research Registry: Genesis, Impact and Future. *COPD* **12 Suppl 1**, 42-5 (2015).
110. Stockley, R.A. et al. Ongoing research in Europe: Alpha One International Registry (AIR) objectives and development. *Eur Respir J* **29**, 582-6 (2007).
111. Chorostowska-Wynimko, J., Struniawski, R., Sliwinski, P., Wajda, B. & Czajkowska-Malinowska, M. The national alpha-1 antitrypsin deficiency registry in Poland. *COPD* **12 Suppl 1**, 22-6 (2015).
112. Stoller, J.K. et al. [American Thoracic Society/European Respiratory Society Statement: Standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency]. *Pneumologie* **59**, 36-68 (2005).
113. Mayer, A.S. et al. Occupational exposure risks in individuals with PI\*Z alpha(1)-antitrypsin deficiency. *Am J Respir Crit Care Med* **162**, 553-8 (2000).
114. Mayer, A.S. et al. Risk factors for symptom onset in PI\*Z alpha-1 antitrypsin deficiency. *Int J Chron Obstruct Pulmon Dis* **1**, 485-92 (2006).
115. Larsson, C. Natural history and life expectancy in severe alpha1-antitrypsin deficiency, Pi Z. *Acta Med Scand* **204**, 345-51 (1978).
116. Strange, C. et al. Genetic testing for alpha1-antitrypsin deficiency. *Genet Med* **6**, 204-10 (2004).
117. Strange, C. et al. Genetic testing of minors for alpha1-antitrypsin deficiency. *Arch Pediatr Adolesc Med* **160**, 531-4 (2006).

118. Piitulainen, E., Tornling, G. & Eriksson, S. Effect of age and occupational exposure to airway irritants on lung function in non-smoking individuals with alpha 1-antitrypsin deficiency (PiZZ). *Thorax* **52**, 244-8 (1997).
119. Eriksson, S., Carlson, J. & Velez, R. Risk of cirrhosis and primary liver cancer in alpha<sub>1</sub>-antitrypsin deficiency. *N. Engl. J. Med.* **314**, 736-739 (1986).
120. Nelson, D.R., Teckman, J., Di Bisceglie, A.M. & Brenner, D.A. Diagnosis and management of patients with alpha<sub>1</sub>-antitrypsin (A1AT) deficiency. *Clin Gastroenterol Hepatol* **10**, 575-80 (2012).
121. He, X.X., Li, Y., Ren, H.P., Tian, D.A. & Lin, J.S. [2010 guideline for the management of hepatocellular carcinoma recommended by the American Association for the Study of Liver Diseases]. *Zhonghua Gan Zang Bing Za Zhi* **19**, 249-50 (2011).
122. Pillai, A.P., Turner, A.M. & Stockley, R.A. Relationship of the 2011 Global Initiative for Chronic Obstructive Lung Disease strategy to clinically relevant outcomes in individuals with alpha<sub>1</sub>-antitrypsin deficiency. *Ann Am Thorac Soc* **11**, 859-64 (2014).
123. Wewers, M.D. et al. Replacement therapy for alpha 1-antitrypsin deficiency associated with emphysema. *N Engl J Med* **316**, 1055-62 (1987).
124. Casolaro, M.A. et al. Augmentation of lung antineutrophil elastase capacity with recombinant human alpha-1-antitrypsin. *J Appl Physiol* (1985) **63**, 2015-23 (1987).
125. Hubbard, R.C., Brantly, M.L., Sellers, S.E., Mitchell, M.E. & Crystal, R.G. Anti-neutrophil-elastase defenses of the lower respiratory tract in alpha 1-antitrypsin deficiency directly augmented with an aerosol of alpha 1-antitrypsin. *Ann Intern Med* **111**, 206-12 (1989).
126. Franciosi, A.N., McCarthy, C. & McElvaney, N.G. The efficacy and safety of inhaled human alpha-1 antitrypsin in people with alpha-1 antitrypsin deficiency-related emphysema. *Expert Rev Respir Med* **9**, 143-51 (2015).
127. Teckman, J.H. & Mangalat, N. Alpha-1 antitrypsin and liver disease: mechanisms of injury and novel interventions. *Expert Rev Gastroenterol Hepatol* **9**, 261-8 (2015).
128. Murray, K.F., Carithers, R.L., Jr. & Aasld. AASLD practice guidelines: Evaluation of the patient for liver transplantation. *Hepatology* **41**, 1407-32 (2005).
129. Tannuri, A.C. et al. Living related donor liver transplantation in children. *Transplant Proc* **43**, 161-4 (2011).
130. Seersholm, N. & Kok-Jensen, A. Survival in relation to lung function and smoking cessation in patients with severe hereditary alpha 1-antitrypsin deficiency. *Am J Respir Crit Care Med* **151**, 369-73 (1995).
131. Tanash, H.A., Nilsson, P.M., Nilsson, J.A. & Piitulainen, E. Clinical course and prognosis of never-smokers with severe alpha-1-antitrypsin deficiency (PiZZ). *Thorax* **63**, 1091-5 (2008).
132. Brantly, M.L. et al. Clinical features and history of the destructive lung disease associated with alpha-1-antitrypsin deficiency of adults with pulmonary symptoms. *Am Rev Respir Dis* **138**, 327-36 (1988).

133. Stoller, J.K. et al. Mortality in individuals with severe deficiency of alpha1-antitrypsin: findings from the National Heart, Lung, and Blood Institute Registry. *Chest* **127**, 1196-204 (2005).
134. Campos, M.A. et al. Clinical characteristics of subjects with symptoms of alpha1-antitrypsin deficiency older than 60 years. *Chest* **135**, 600-8 (2009).
135. Needham, M. & Stockley, R.A. Alpha 1-antitrypsin deficiency. 3: Clinical manifestations and natural history. *Thorax* **59**, 441-5 (2004).
136. group, T.a.-.-a.d.r.s. Survival and FEV<sub>1</sub> decline in individuals with severe deficiency of  $\alpha_1$ -antitrypsin. *Am. J. Respir. Crit. Care Med.* **158**, 49-59 (1998).
137. Dawkins, P.A., Dowson, L.J., Guest, P.J. & Stockley, R.A. Predictors of mortality in alpha1-antitrypsin deficiency *Thorax* **58**, 1020-1026 (2003).
138. Demeo, D.L. et al. Determinants of airflow obstruction in severe alpha-1-antitrypsin deficiency. *Thorax* **62**, 806-13 (2007).
139. Holm, K.E. et al. Differences in adjustment between individuals with alpha-1 antitrypsin deficiency (AATD)-associated COPD and non-AATD COPD. *COPD* **10**, 226-34 (2013).
140. Campos, M.A., Alazemi, S., Zhang, G., Wanner, A. & Sandhaus, R.A. Effects of a disease management program in individuals with alpha-1 antitrypsin deficiency. *COPD* **6**, 31-40 (2009).
141. Dirksen, A. et al. Exploring the role of CT densitometry: a randomised study of augmentation therapy in alpha1-antitrypsin deficiency. *Eur Respir J* **33**, 1345-53 (2009).
142. Chapman, K.R. et al. Intravenous augmentation treatment and lung density in severe alpha1 antitrypsin deficiency (RAPID): a randomised, double-blind, placebo-controlled trial. *Lancet* **386**, 360-8 (2015).
143. Rashid, S.T. et al. Modeling inherited metabolic disorders of the liver using human induced pluripotent stem cells. *J Clin Invest* **120**, 3127-36 (2010).
144. Tafaleng, E.N. et al. Induced pluripotent stem cells model personalized variations in liver disease resulting from alpha1-antitrypsin deficiency. *Hepatology* **62**, 147-57 (2015).
145. Dirksen, A. et al. A randomized clinical trial of alpha(1)-antitrypsin augmentation therapy. *Am J Respir Crit Care Med* **160**, 1468-72 (1999).
146. Kaushal, S. et al. Rapamycin reduces intrahepatic alpha-1-antitrypsin mutant Z protein polymers and liver injury in a mouse model. *Exp Biol Med (Maywood)* **235**, 700-9 (2010).
147. Mahadeva, R., Dafforn, T.R., Carrell, R.W. & Lomas, D.A. 6-mer peptide selectively anneals to a pathogenic serpin conformation and blocks polymerization. Implications for the prevention of Z alpha(1)-antitrypsin-related cirrhosis. *J Biol Chem* **277**, 6771-4 (2002).
148. Guo, S. et al. Antisense oligonucleotide treatment ameliorates alpha-1 antitrypsin-related liver disease in mice. *J Clin Invest* **124**, 251-61 (2014).
149. Burrows, J.A., Willis, L.K. & Perlmutter, D.H. Chemical chaperones mediate increased secretion of mutant alpha 1-antitrypsin (alpha 1-AT) Z: A potential pharmacological strategy for prevention of liver injury and emphysema in alpha 1-AT deficiency. *Proc Natl Acad Sci U S A* **97**, 1796-801 (2000).

150. Teckman, J.H. Lack of effect of oral 4-phenylbutyrate on serum alpha-1-antitrypsin in patients with alpha-1-antitrypsin deficiency: a preliminary study. *J Pediatr Gastroenterol Nutr* **39**, 34-7 (2004).
151. Balch, W.E., Morimoto, R.I., Dillin, A. & Kelly, J.W. Adapting proteostasis for disease intervention. *Science* **319**, 916-9 (2008).
152. Roth, D.M. et al. Modulation of the maladaptive stress response to manage diseases of protein folding. *PLoS Biol* **12**, e1001998 (2014).
153. Bouhcecareilh, M., Hutt, D.M., Szajner, P., Flotte, T.R. & Balch, W.E. Histone deacetylase inhibitor (HDACi) suberoylanilide hydroxamic acid (SAHA)-mediated correction of alpha1-antitrypsin deficiency. *J Biol Chem* **287**, 38265-78 (2012).
154. Parfrey, H., Dafforn, T.R., Belorgey, D., Lomas, D.A. & Mahadeva, R. Inhibiting polymerization: new therapeutic strategies for Z alpha1-antitrypsin-related emphysema. *Am J Respir Cell Mol Biol* **31**, 133-9 (2004).
155. Nyon, M.P. & Gooptu, B. Therapeutic targeting of misfolding and conformational change in alpha1-antitrypsin deficiency. *Future Med Chem* **6**, 1047-65 (2014).
156. Chang, Y.P. et al. Targeting serpins in high-throughput and structure-based drug design. *Methods Enzymol* **501**, 139-75 (2011).
157. Chang, Y.P., Mahadeva, R., Chang, W.S., Lin, S.C. & Chu, Y.H. Small-molecule peptides inhibit Z alpha1-antitrypsin polymerization. *J Cell Mol Med* **13**, 2304-16 (2009).
158. Nyon, M.P. et al. An integrative approach combining ion mobility mass spectrometry, X-ray crystallography, and nuclear magnetic resonance spectroscopy to study the conformational dynamics of alpha1 -antitrypsin upon ligand binding. *Protein Sci* **24**, 1301-12 (2015).
159. Gooptu, B. et al. Crystallographic and cellular characterisation of two mechanisms stabilising the native fold of alpha1-antitrypsin: implications for disease and drug design. *J Mol Biol* **387**, 857-68 (2009).
160. Mallya, M. et al. Small molecules block the polymerization of Z alpha1-antitrypsin and increase the clearance of intracellular aggregates. *J Med Chem* **50**, 5357-63 (2007).
161. Patschull, A.O., Gooptu, B., Ashford, P., Daviter, T. & Nobeli, I. In silico assessment of potential druggable pockets on the surface of alpha1-antitrypsin conformers. *PLoS One* **7**, e36612 (2012).
162. Loring, H.S. & Flotte, T.R. Current status of gene therapy for alpha-1 antitrypsin deficiency. *Expert Opin Biol Ther* **15**, 329-36 (2015).
163. Flotte, T.R. et al. Phase 2 clinical trial of a recombinant adeno-associated viral vector expressing alpha1-antitrypsin: interim results. *Hum Gene Ther* **22**, 1239-47 (2011).
164. Chulay, J.D. et al. Preclinical evaluation of a recombinant adeno-associated virus vector expressing human alpha-1 antitrypsin made using a recombinant herpes simplex virus production method. *Hum Gene Ther* **22**, 155-65 (2011).
165. Brantly, M.L. et al. Sustained transgene expression despite T lymphocyte responses in a clinical trial of rAAV1-AAT gene therapy. *Proc Natl Acad Sci U S A* **106**, 16363-8 (2009).

166. Brantly, M.L. et al. Phase I trial of intramuscular injection of a recombinant adeno-associated virus serotype 2 alpha1-antitrypsin (AAT) vector in AAT-deficient adults. *Hum Gene Ther* **17**, 1177-86 (2006).
167. Liqun Wang, R. et al. Recombinant AAV serotype and capsid mutant comparison for pulmonary gene transfer of alpha-1-antitrypsin using invasive and noninvasive delivery. *Mol Ther* **17**, 81-7 (2009).
168. Mueller, C. et al. Human Treg responses allow sustained recombinant adeno-associated virus-mediated transgene expression. *J Clin Invest* **123**, 5310-8 (2013).
169. Ghaedi, M., Lotfi, A.S. & Soleimani, M. Establishment of lentiviral-vector-mediated model of human alpha-1 antitrypsin delivery into hepatocyte-like cells differentiated from mesenchymal stem cells. *Tissue Cell* **42**, 181-9 (2010).
170. Argyros, O. et al. Persistent episomal transgene expression in liver following delivery of a scaffold/matrix attachment region containing non-viral vector. *Gene Ther* **15**, 1593-605 (2008).
171. Chiuchiolo, M.J. et al. Phase I/II study of intrapleural administration of a serotype rh.10 replication-deficient adeno-associated virus gene transfer vector expressing the human alpha1-antitrypsin cDNA to individuals with alpha1-antitrypsin deficiency. *Hum Gene Ther Clin Dev* **25**, 112-33 (2014).
172. Yusa, K. et al. Targeted gene correction of alpha1-antitrypsin deficiency in induced pluripotent stem cells. *Nature* **478**, 391-4 (2011).
173. Wilson, A.A. et al. Emergence of a stage-dependent human liver disease signature with directed differentiation of alpha-1 antitrypsin-deficient iPS cells. *Stem Cell Reports* **4**, 873-85 (2015).
174. Roussel, B.D. et al. Endoplasmic reticulum dysfunction in neurological disease. *Lancet Neurol* **12**, 105-18 (2013).
175. Griese, M. et al. alpha1-Antitrypsin inhalation reduces airway inflammation in cystic fibrosis patients. *Eur Respir J* **29**, 240-50 (2007).
176. McElvaney, N.G. et al. Aerosol alpha 1-antitrypsin treatment for cystic fibrosis. *Lancet* **337**, 392-4 (1991).
177. Pahl, H.L. & Baeuerle, P.A. A novel signal transduction pathway from the endoplasmic reticulum to the nucleus is mediated by transcription factor NF-kappa B. *EMBO J* **14**, 2580-8 (1995).
178. Pahl, H.L. & Baeuerle, P.A. Expression of influenza virus hemagglutinin activates transcription factor NF-kappa B. *J Virol* **69**, 1480-4 (1995).
179. Sveger, T.  $\alpha_1$ -antitrypsin deficiency in early childhood. *Pediatrics* **62**, 22-25 (1978).
180. Sveger, T. The natural history of liver disease in  $\alpha_1$ -antitrypsin deficient children. *Acta Paed. Scand.* **77**, 847-51 (1988).
181. Sveger, T. & Eriksson, S. The liver in adolescents with  $\alpha_1$ -antitrypsin deficiency. *Hepatology* **22**, 514-517 (1995).
182. Eden, E. et al. Atopy, asthma, and emphysema in patients with severe alpha-1-antitrypsin deficiency. *Am J Respir Crit Care Med* **156**, 68-74 (1997).
183. Franciosi, A.N., McCarthy, C., Carroll, T.P. & McElvaney, N.G. Unusual Acute Sequelae of alpha1-Antitrypsin Deficiency: A Myriad of Symptoms With One Common Cure. *Chest* **148**, e136-8 (2015).

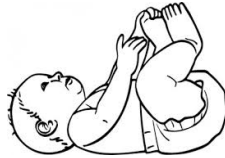
184. Elzouki, A.N., Segelmark, M., Wieslander, J. & Eriksson, S. Strong link between the alpha 1-antitrypsin PiZ allele and Wegener's granulomatosis. *J Intern Med* **236**, 543-8 (1994).
185. Ciliberto, G., Dente, L. & Cortese, R. Cell-specific expression of a transfected human alpha 1-antitrypsin gene. *Cell* **41**, 531-40 (1985).
186. Rollini, P. & Fournier, R.E. Differential regulation of gene activity and chromatin structure within the human serpin gene cluster at 14q32.1 in macrophage microcell hybrids. *Nucleic Acids Res* **28**, 1767-77 (2000).
187. Seersholm, N. et al. Does alpha1-antitrypsin augmentation therapy slow the annual decline in FEV1 in patients with severe hereditary alpha1-antitrypsin deficiency? Wissenschaftliche Arbeitsgemeinschaft zur Therapie von Lungenerkrankungen (WATL) alpha1-AT study group. *Eur Respir J* **10**, 2260-3 (1997).
188. Lieberman, J. Augmentation therapy reduces frequency of lung infections in antitrypsin deficiency: a new hypothesis with supporting data. *Chest* **118**, 1480-5 (2000).
189. Wencker, M., Fuhrmann, B., Banik, N., Konietzko, N. & Wissenschaftliche Arbeitsgemeinschaft zur Therapie von, L. Longitudinal follow-up of patients with alpha(1)-protease inhibitor deficiency before and during therapy with IV alpha(1)-protease inhibitor. *Chest* **119**, 737-44 (2001).
190. Tonelli, A.R., Rouhani, F., Li, N., Schreck, P. & Brantly, M.L. Alpha-1-antitrypsin augmentation therapy in deficient individuals enrolled in the Alpha-1 Foundation DNA and Tissue Bank. *Int J Chron Obstruct Pulmon Dis* **4**, 443-52 (2009).

Figure 1.

### Liver disease in PiZZ Children

Cholestatic Jaundice (10%)  
*of which 15% develop Juvenile Cirrhosis*  
Other liver disease (6%)

- Z polymers form in ER of hepatocytes *in utero*
- Raised serum alanine transferase at 1 year, decreasing by age 12
- Raised serum bilirubin
- Risk of death 2-3%



### Lung disease in PiZZ adults

Emphysema: basal, panlobar

Deterioration associated with

- current smoking, age 30 to 44 years
- male sex
- FEV<sub>1</sub> between 35 to 60% predicted

- asthmatic features

### Liver manifestations in <50% PiZZ adults

Cirrhosis, Hepatocellular carcinoma

Risk factors: Male sex and obesity

Figure 2.

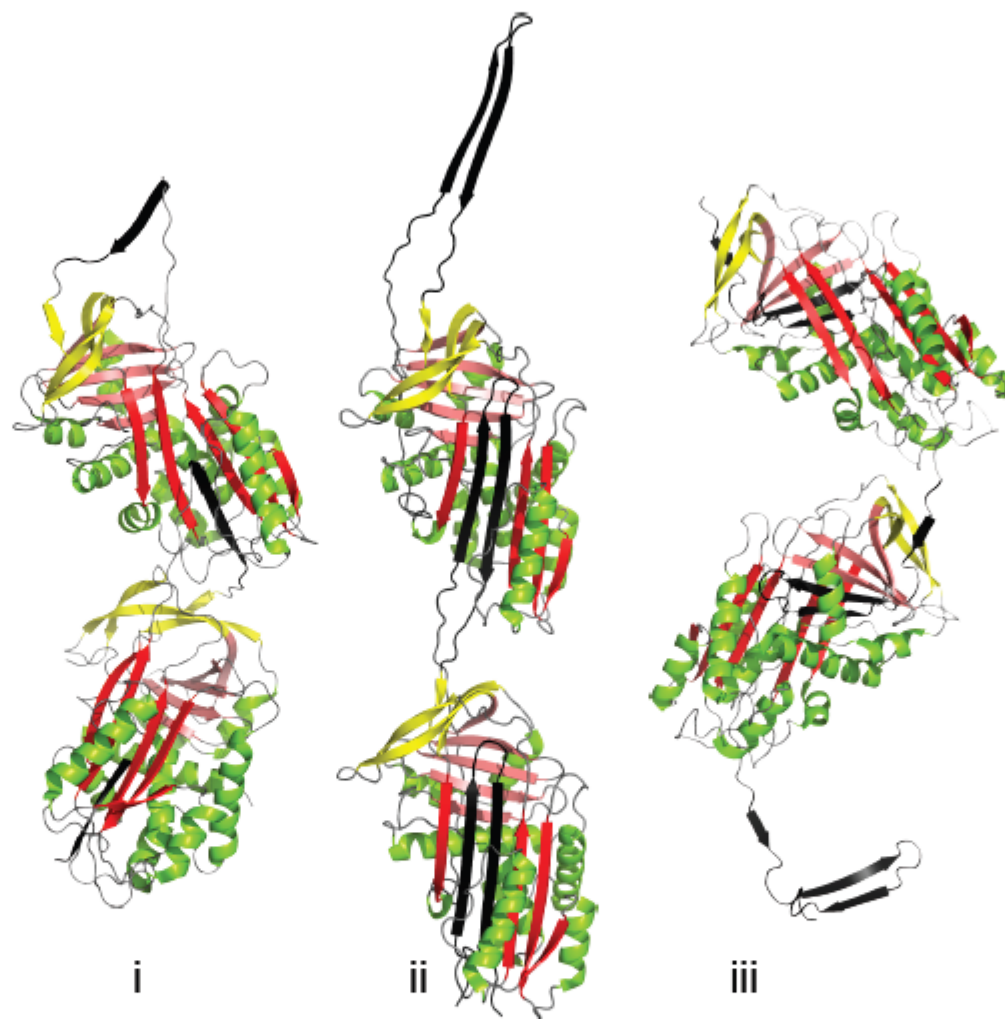




Figure 3.

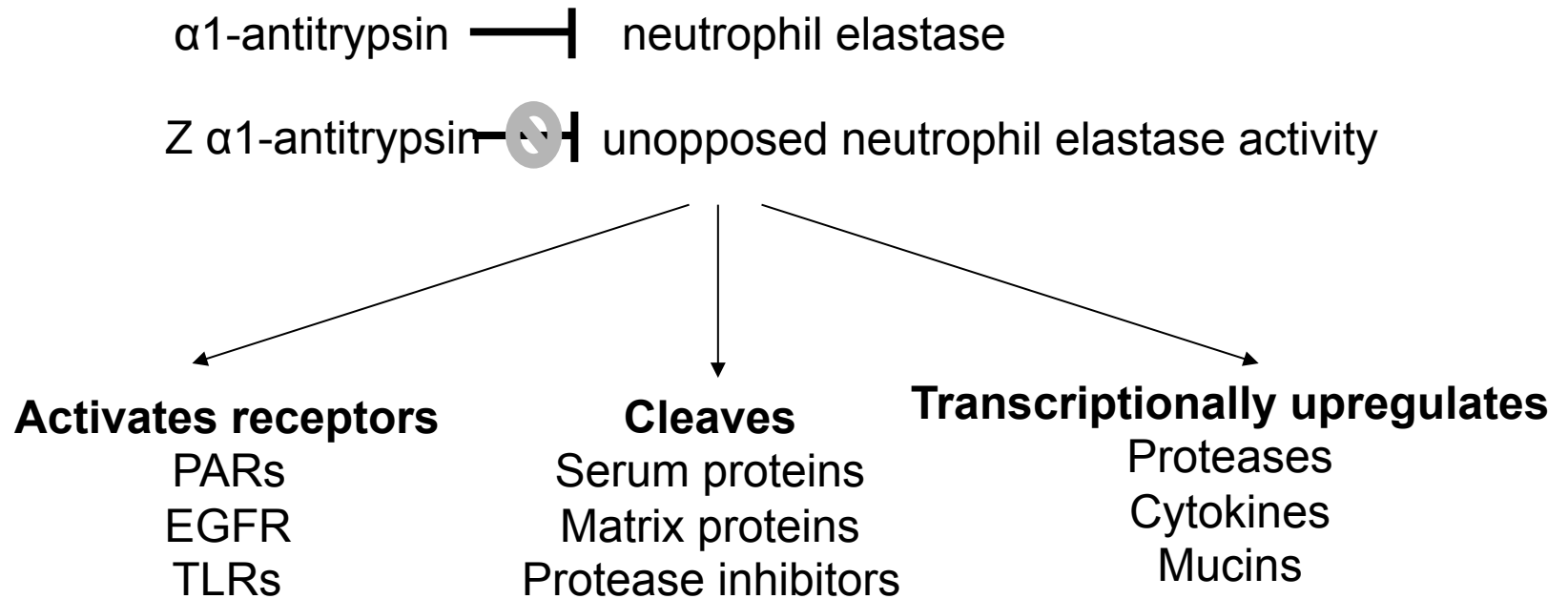


Figure 3.

$\alpha$ 1-antitrypsin —| neutrophil elastase

Z  $\alpha$ 1-antitrypsin —| unopposed neutrophil elastase activity

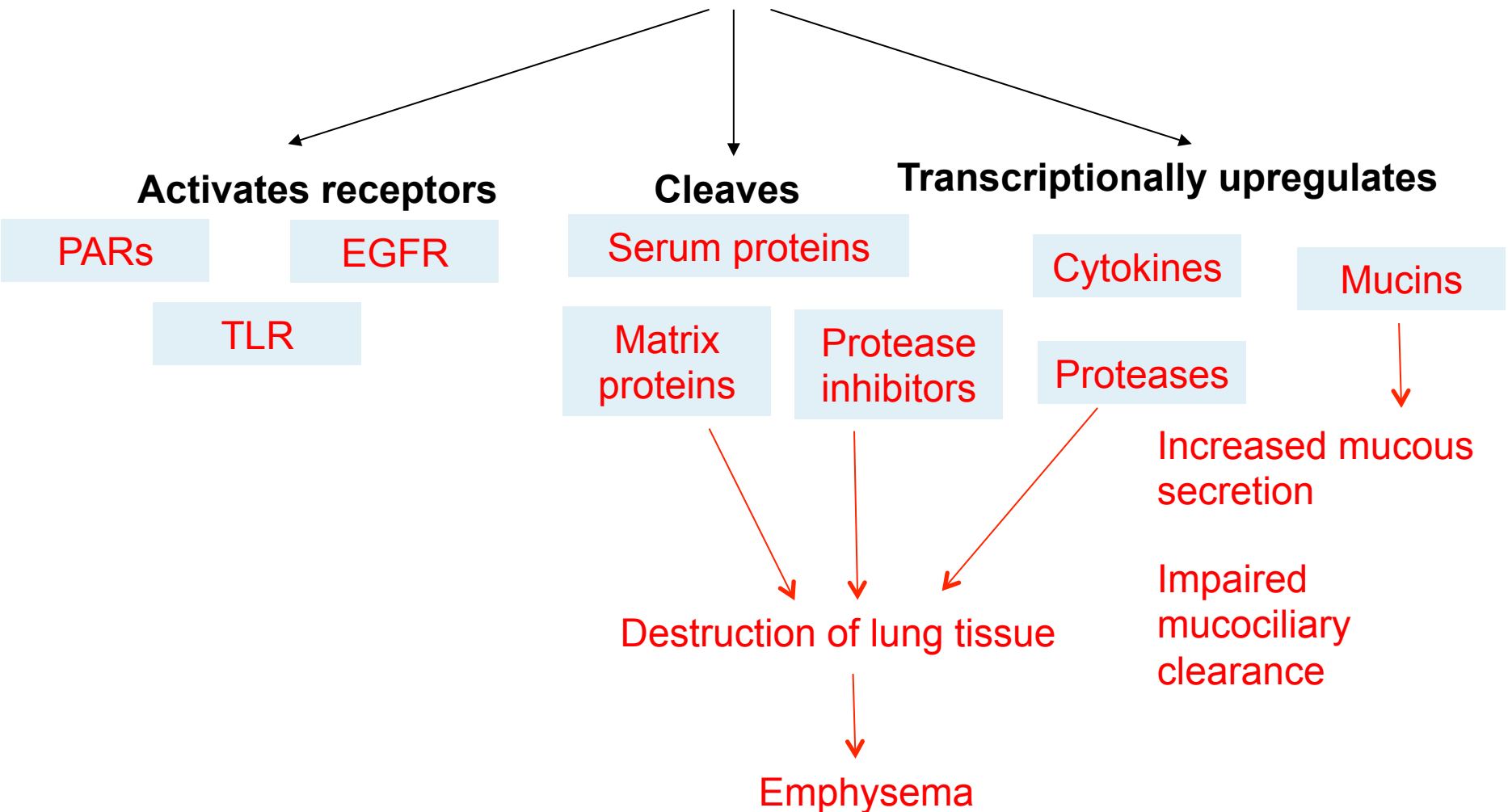


Figure 4. Similar content to this, but needs to be redrawn please

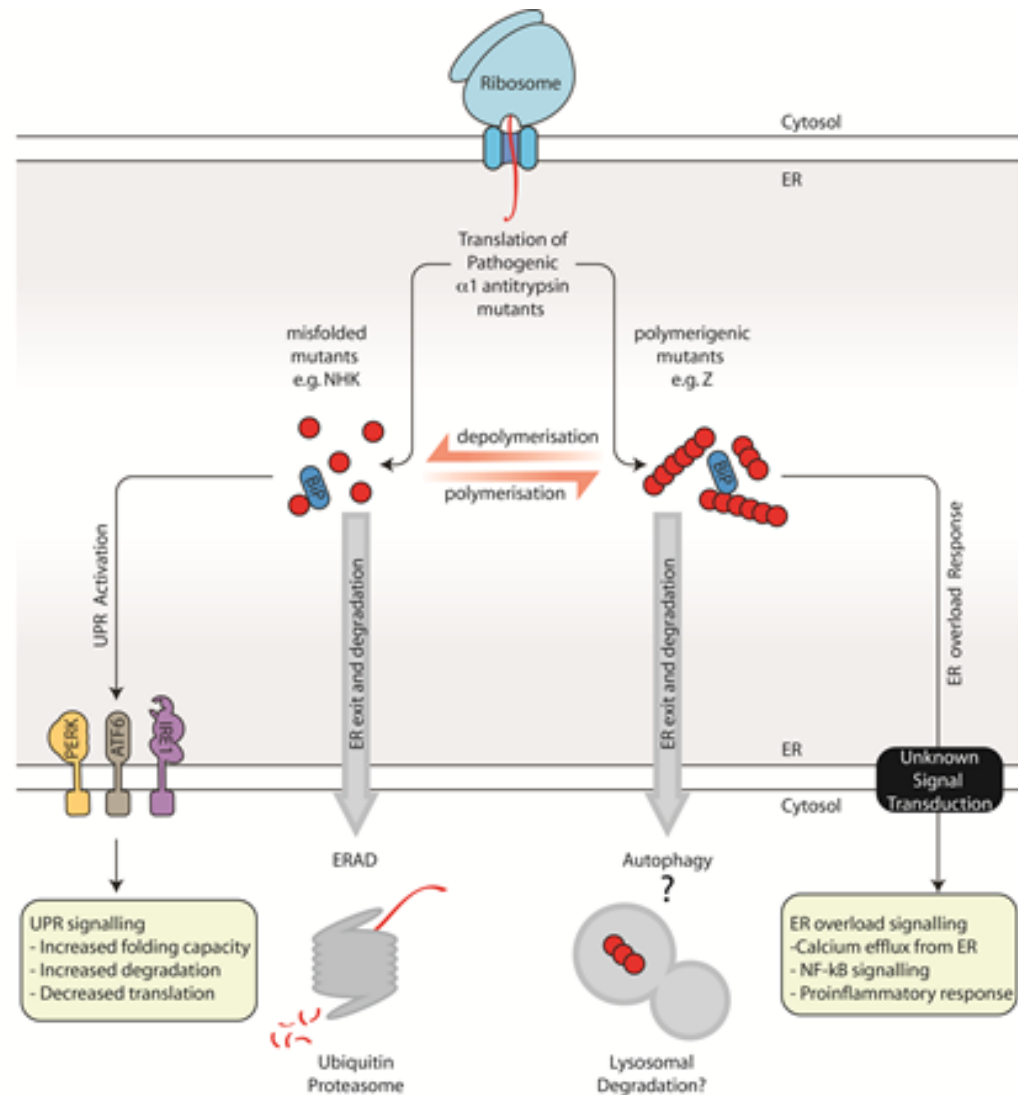


Figure 5.

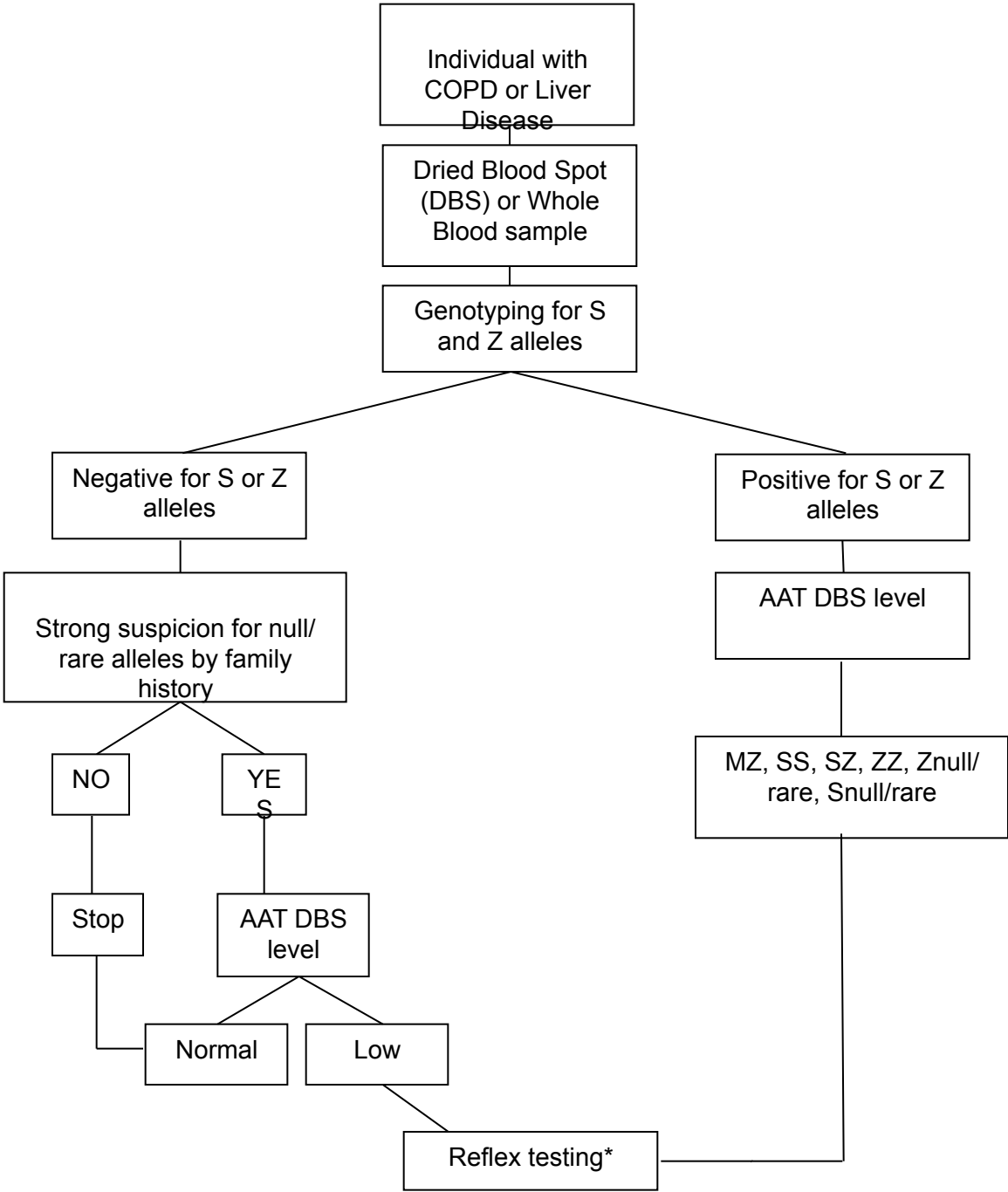
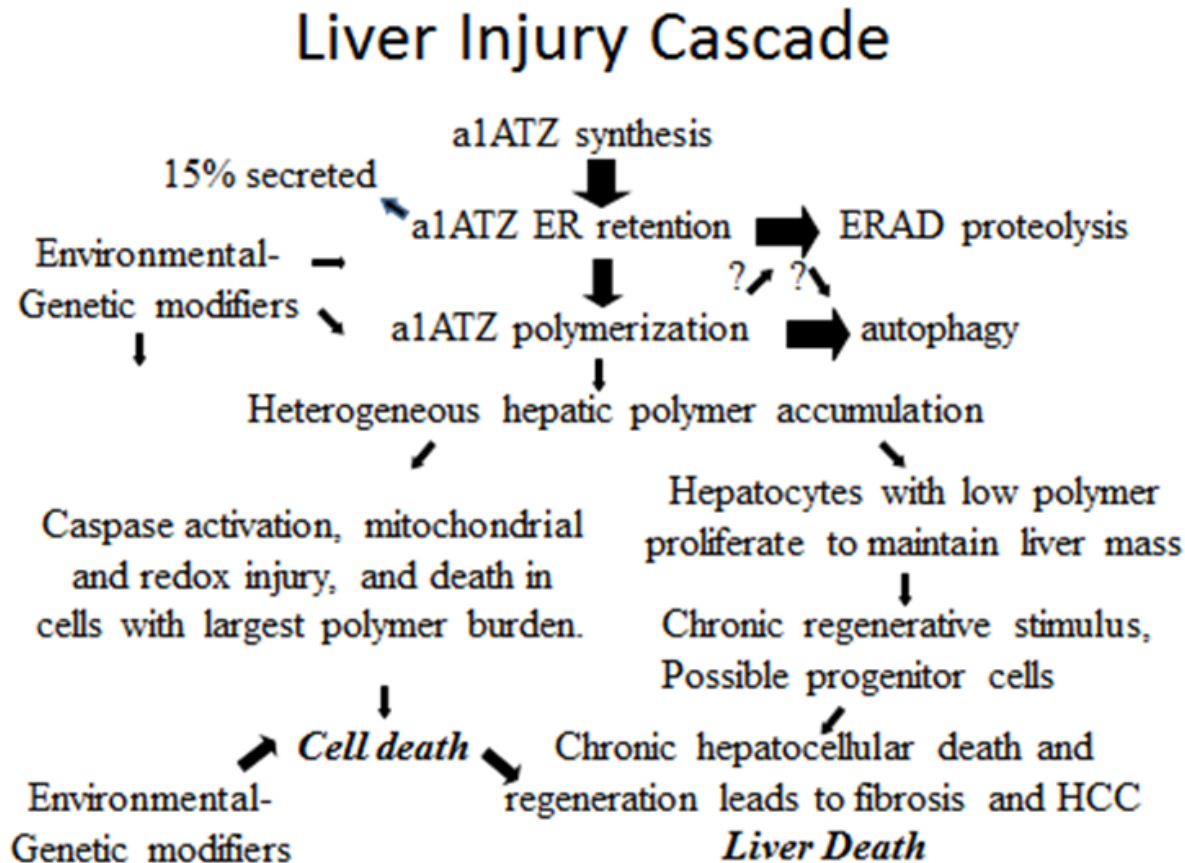


Figure 6. Can this be converted into a diagram, please



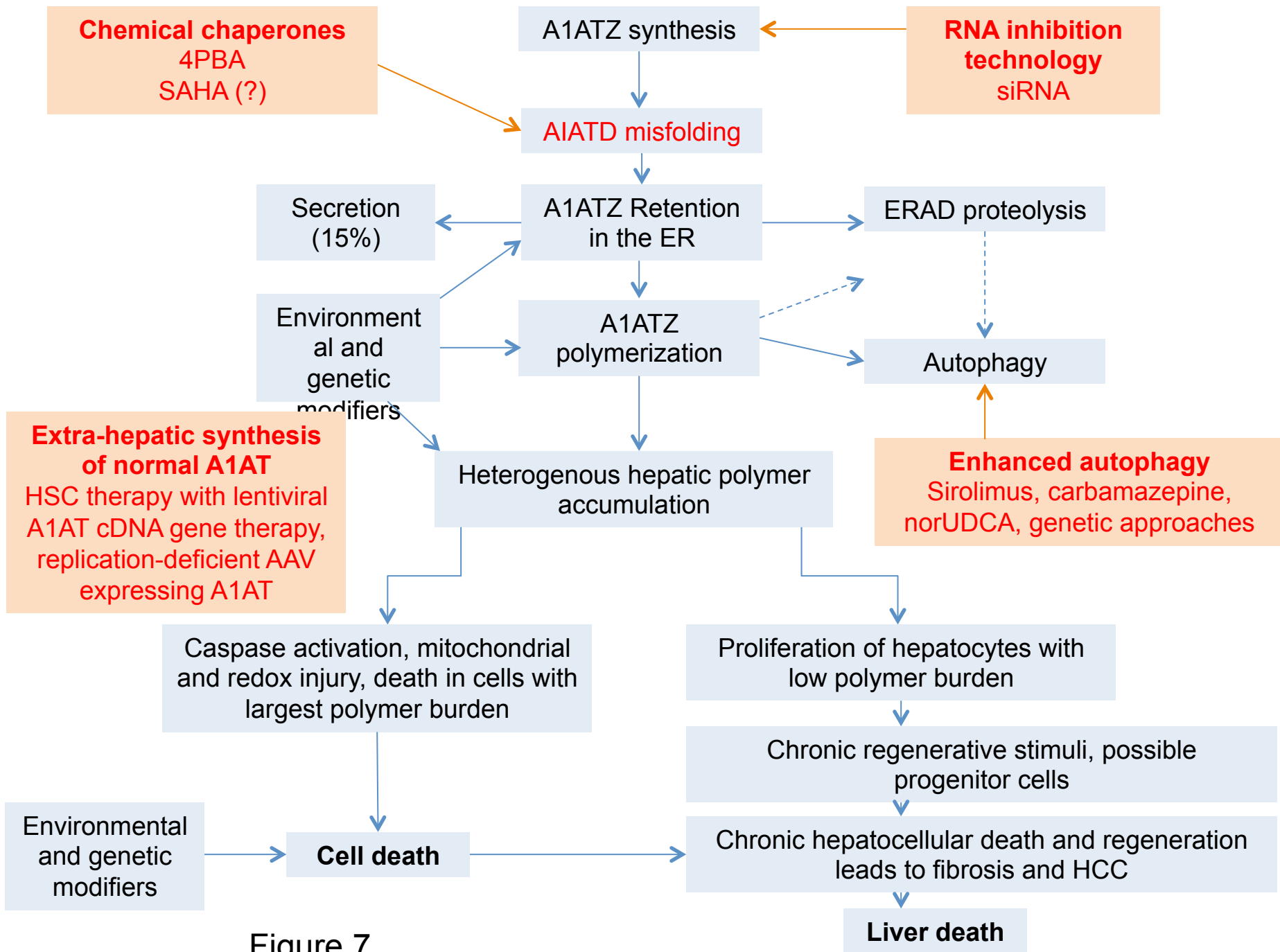


Figure 7.